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# Detecting evidence of systemic inflammation from osteological markers in the Indian Knoll population of Ohio county, Kentucky.

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# DETECTING EVIDENCE OF SYSTEMIC INFLAMMATION FROM OSTEOLOGICAL MARKERS IN THE INDIAN KNOLL POPULATION OF OHIO COUNTY, KENTUCKY

By

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B.A., Northern Kentucky University, 2011

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ABSTRACT

DETECTING EVIDENCE OF SYSTEMIC INFLAMMATION FROM OSTEOLOGICAL MARKERS IN THE INDIAN KNOLL POPULATION OF OHIO COUNTY, KENTUCKY

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Indian Knoll is an Archaic shell midden site located in the Green River region of west-central Kentucky, and was excavated twice in the first half of the $20^{th}$ century. While Indian Knoll has been the subject of frequent bioarchaeological studies, the present study presents an osteological analysis of the relationships between skeletal markers commonly associated with local inflammatory processes, and that have the potential to be used as proxies for systemic inflammation. The analysis revealed a significant positive association in the presence of periodontal disease (PD) and periosteal lesions (PL), suggesting a potential underlying hyper-inflammatory status or phenotype. These findings may indicate that different stress factors could have induced a shift in systemic inflammation. Significant positive associations were also observed between PD and age, and PL and age, which indicate an age effect. The present study also explored the associations between additional inflammatory lesions, and comparisons between age and sex.

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# INTRODUCTION

Bioarchaeology relies on the skeletal remains of human populations to reconstruct and interpret past lifeways, patterns of disease, and states of relative health across time and space. In ideal circumstances, the preservation of human skeletal remains is optimal and a thorough analysis can be conducted on each of these aspects. One such population exists in the state of Kentucky: Indian Knoll is one of the largest Archaic collections in the state, and thus has been the center of a number of studies related to health and disease.

With ever changing ideas in our understanding of disease, its manifestations, and a wealth of information from the archaeological context, including timelines, site use, and social organization, we can continue to shed new light on previously studied populations and build upon the groundwork that has been laid. Recent studies have searched for evidence of a hyper-inflammatory phenotype capable of inducing systemic infection (e.g. Crespo *et al.* 2016; Moutsopoulos and Madianos 2006; Shaddox *et al.* 2010). Previous studies of health and disease at Indian Knoll (e.g. Cassidy 1972 and Kelley 1980) have demonstrated a number of bone lesions, in addition to nutritional stress markers, indicative of inflammation. The present study sought to rescore and reanalyze a number of these previously scored bone lesions, and uncovered their associations and reliability as proxies for detecting systemic inflammation.

# OBJECTIVE & HYPOTHESIS

The primary objective of this research project was to conduct an osteological analysis of the relationships among skeletal lesions that are commonly associated with local inflammatory processes, and that have the potential to be used as proxies for systemic inflammation.

Several markers of inflammation and nutritional deficiencies were recorded per individual, and age and sex was estimated. The lesions of primary importance to this analysis were periodontal disease and periosteal lesions, with secondary lesions including cribra orbitalia, porotic hyperostosis, abscessing, enamel hypoplasia, tibial bowing, and auditory exostoses. Individual lesions were compared using chi-square tests, and lesions were compared to age categories and sex estimations. The chi-square analyses that were performed are summarized in Table 1. The three-way association between periodontal disease, periosteal lesions, and age was tested using hierarchical log linear analysis.

The present study examined a sample of skeletons that was pseudo-random, and lesions were scored for presence and severity on specified elements of each individual sampled. All data were recorded in a database, and transferred to a spreadsheet and recoded for statistical analyses. All analyses were performed in version 22 of SPSS.

A similar research design has been carried out on a different population (DeWitte and Bekvalac 2011). Hence, similar associations were expected in the Indian Knoll population. The anticipated results for this analysis were:

1) Evidence of an association between infectious inflammatory conditions such as periodontal disease and periosteal lesions, in addition to periodontal disease and abscessing;

2) Evidence of an association between periosteal lesions and bowing of the tibiae;

3) Evidence of an association between markers indicative of nutritional deficiencies, specifically those lesions associated with cribra orbitalia and porotic hyperostosis, since they may be of the same etiology; or

4) No evidence of an association between inflammatory lesions indicative of infection and lesions indicative of nutritional deficiencies, metabolic, or hematological disorders.

It was postulated that if the first hypothesis proved true, the inflammatory lesions in this population may be the result of a hyper-inflammatory trait capable of producing an over-reactive immune response. Additionally, because bowing of the tibiae can be the result of either a metabolic and infectious condition, the associations detected between these conditions may lead to a diagnosis of the bowing.

Regardless of the results, this analysis was expected to shed light on the human immune response to multiple inflammatory lesions. In this study, the osteological markers were not considered as generators of hyper-inflammation, per se. Bone lesions, where inflammation was present, and the associations discovered, were used to detect systemic inflammation but not to explain the complex etiology of an over-reactive inflammatory response.

| | **AB** | **Bow** | **CO** | **EH** | **Exo** | **PD** | **PH** | **PL** | **Age** | **Sex** |
|---|---|---|---|---|---|---|---|---|---|---|
| **AB** | X | X | X | X | X | | X | | | |
| **Bow** | | X | | X | X | X | | | X | |
| **CO** | | | X | | | | | | | |
| **EH** | | | | X | | | | | | |
| **Exo** | | | | | X | | | | | |
| **PD** | | | | | | X | | | | |
| **PH** | | | | | | | X | | | |
| **PL** | | | | | | | | X | | |
| **Age** | | | | | | | | | X | X |
| **Sex** | | | | | | | | | | X |

Table 1. Table showing the interactions that were tested in chi-squares.

**KEY:**
AB = Abscessing
Bow = Tibial bowing
CO = Orbital porosity
EH = Enamel hypoplasia
Exo = Auditory exostoses
PD = Periodontal disease
PH = Cranial porosity
PL = Periosteal lesions
X = Test not performed

## INDIAN KNOLL: THE SITE

Indian Knoll (15Oh2) is an archaeological site consisting of a shell midden in Ohio County, Kentucky, located along the Green River in west-central Kentucky. The Green River is a tributary of the Ohio River, and the middle portion serves as the drainage system for caverns in Mammoth Cave, Kentucky. The region was described by a number of early American settlers to the region as being lush with fertile land, pockets of dense hardwood forest, and the river good for fishing with its deep waters (Stein 2005:20), which may be beneficial in our understanding of why people ultimately inhabited the site thousands of years ago.

Initial excavations of the site took place in 1915 and were led by C.B. Moore, during which 298 skeletons were exhumed (Webb 1946:121). In 1939 excavations of the site began again, and were supervised by Marion Baugh (Webb 1946:115-116). Though Baugh and his crew of Works Progress Administration (WPA) workers excavated a large portion of Indian Knoll and unearthed over 880 skeletons (Snow 1948:385-386), portions around the periphery of the site remain unexplored and unexcavated (Morey *et al.*2002:548; Webb 1946:125).

Roughly elliptical in shape, the Indian Knoll site measures 450 feet long by 220 feet wide (roughly 137.2 by 67.1 m) with depths that ranged from five feet to eight feet (roughly 1.5 to 2.4 m) (Webb 1946:119). Over the number of years that the site was utilized, the accumulation of mussel shells across the site led to the formation of what is referred to as a shell mound (Webb 1946:115). These mounds have also been referred to as shell middens and shell heaps (Hensley 1994:13).

There are many archaeological sites along the Green River in Kentucky, a number of them shell mounds. These shell mounds are made up of a number of materials from cultural habitation, but shell does not comprise the majority, by volume, at any site (Marquardt and Watson 2005:632). Shell middens are not unique to the Green River Region of Kentucky, however, as there are hundreds of sites across the southeast and Midwest United States: the trend continues north at the confluence of the Ohio and Little Miami rivers in Ohio, and south to the Eva site situated on the Tennessee River in Benton county, Tennessee, the Savannah River in Georgia, St. Johns River in Florida (Claassen 1992:1, 2005:279), and in Alabama (Snow 1948:531).

*Skeletal Population*

Although the site produced 1,234 skeletons, this number is problematic. Eight-hundred and eighty skeletons were recorded to have been exhumed in the WPA excavation led by Baugh in the 1930s (Webb 1946:173), and prior to that 298 skeletons were unearthed by C. B. Moore in the excavations of 1915 (Webb 1946:121). The remaining skeletons included infants, children, and youths (Snow 1948:385-386). It was discovered in the second excavation that Moore had apparently removed the crania and left the less ideal postcranial remains in the field; the remains were excavated by Baugh and labeled “Disturbed Area Debris” (Snow 1948:384; Webb 1046:127). It was stated by Webb that Moore sent 66 skeletons to the National Museum after the first excavation of the site (Webb 1946:127). The majority of skeletons exhumed at Indian Knoll are curated at the William S. Webb Museum of Anthropology at the University of Kentucky in Lexington (Snow 1948:386).

*Temporal Span of Indian Knoll*

The radiocarbon dates available for Indian Knoll indicate a primarily mid to late Archaic settlement, with minimal deposition continuing into the Woodland period. Though evidence exists for continued occupation into the Woodland period, we must not assume that the site was continually occupied through the late Archaic and Woodland periods. It is noted by Morey *et al.* (2002:539) that the Green River sites continued to be occupied at least sporadically after the discontinuation of shell deposits. However, there were few artifacts deposited at Indian Knoll in the Woodland period (Morey *et al.* 2002:539).

Early radiocarbon dates from different levels place the date of occupation at 4282 ± 250 uncalibrated years B.P., and 3963 ± 350 uncalibrated years B.P. (Libby 1952:116). More recently, calibrated dates on charred nutshells (Morey *et al.* 2002:538) indicated a starting range between 5590 to 4560 cal years B.P., with the end range spanning between 5420 to 4470 cal years B.P., with each date occurring at a different depth. Webb believed that Indian Knoll was occupied for about 500 years (Snow 1948:387), and Kelley (1980:32) postulated that it was likely occupied for 1200-1300 years. Herrmann (2002:73) postulated that the period of deposition could be as few as 500 years given the overlap of radiocarbon dates from various depths within the site, a fact that is also noted by Morey *et al.* (2002:539).

An analysis by Rolingson (1967) of the stylistic qualities of artifacts from Indian Knoll, which included ceramics and stone tools, seems to indicate that the site was used primarily during the mid to late Archaic period, but that habitation may have extended into the early Woodland period (1967:398-400). This fact correlates with the calibrated radiocarbon dates reported above. Other Green River sites indicate a similar pattern based on artifact analysis (Morey *et al.* 2002:O539).

*Site Formation and Utilization*

Shell deposits closest to the river measured depths of eight feet (2.4 m), whereas a second bank of the site contained cultural deposits five feet (1.5 m) in depth (Webb 1946:119). According to Webb, there was no evidence of soil having been brought to the site to cover the detritus deposited by its inhabitants, but two areas of clay are thought to represent the floors created for use during habitation (Webb 1946:119, 129). Only six fire pits were found throughout the site (Webb 1946:129), and features throughout the site are few in number (Webb 1946:129), adding to the debate over the purpose the site held for its prehistoric inhabitants. The stratigraphy of some Green River Archaic sites is composed of a shell-laden zone, and on top of it a shell-free zone with similar cultural artifacts; thus, individuals continued to be buried in these shell-free zones when shell was no longer being deposited (Claassen 1992:9).

Several schools of thought exist over the formation of Indian Knoll and whether it was used as a seasonal or permanent habitation site, and how the site was utilized. Perhaps the most widely supported hypothesis is that the site use was seasonal (Cassidy 1972:84; Claassen 2005:291, Claassen 2010:9, 196; Crothers 1999:249; Hensley 1994:239,254; Kelley 1980:32; Rolingson 1967:395-396; Marquardt and Watson 2005:637). A lack of abundant features (Webb 1946:129), specifically hearths, has been used as evidence that the site may not have been utilized on a year-round basis (Claassen 1992:5, 8; Rolingson 1967:395).

A theory of site formation developed by Cheryl Claassen (1992) proposes that the mound was intentionally created and laid with shell as part of a ceremonial process (1992; 2010), a hypothesis that Hensley (1994:251) discredits on the claim that there is little evidence to support it. Under Claassen's hypothesis (1992:1), the mounded shell was used for human burials despite the fact that there is little ethnographic data from the eastern United States to indicate a symbolic use of shell (Claassen 1992:3). Claassen (1992:5, 8) cites the low prevalence of features as positive evidence against the hypothesis that Indian Knoll was a year-round base camp, especially considering that there is no evidence of a housing structure at the site. Woodland burial mounds containing shell have rarely been documented (Claassen 1992:2), suggesting that this was strictly an Archaic practice. Adding to her hypothesis, Claassen noted that shell artifacts are the most common materials associated with burials at Indian Knoll, and most grave goods in shell mound burials are made of shell (Claassen 1992:5). However, negative evidence for a ceremonial purpose of mollusk shells does lend itself to the argument. The primary type of refuse at the site lends itself to the hypothesis that the site was a habitation site of sorts (Claassen 1992:8).

Claassen proposes the idea that men of the Green River region may have been keepers of the river, and that constant exposure to the cold waters may have led to the formation of auditory exostoses (2010:191). Auditory exostoses are common in males at Indian Knoll, which will be discussed in greater detail later. Additionally, Claassen (2010) postulated the mortuary importance of shell at the Green River sites, adding that shellfish were an important component of the sites as a food source (Claassen 2010:9). She suggested that the shells may have been consumed in public feasts when captives and relatives were buried, even proposing the possibility of shells having renewing properties when they were interred with the bodies (Claassen 2010:196). However, Claassen (2010:122-124) hypothesized a mortuary use of some Green River sites, such as Indian Knoll, based on the presence of a consecrating burial at the deepest cultural level of the sites. This hypothesis posits that these sites may have started as villages and were later used as mortuaries, hence the consecrating burial, although just the opposite could also hold true (Claassen 2010:124).

We must concede that mortuary activity at Indian Knoll is certainly something of a puzzle. It is possible that the degree of difference between artifacts in burials is associated with individual roles in the society (Haskins and Herrmann 1996:113-114). For example, Winters (1968:215) hypothesized that the presence of marine shell from coastal regions in some burials may be indicative of status items. Rothschild (1979:671-672) analyzed clusters of burials and the goods included in each, ultimately reaching the conclusion that Indian Knoll was less purely egalitarian than expected, with some evidence of possible social stratification. A recent analysis of burials, grave goods, and musculoskeletal stress markers demonstrated differences in associations between the sexes, some of which may be indicative of the activities, or lack thereof, performed by individuals (Nagy 2000:278-279). Marquardt and Watson (2005:637-638) acknowledge the relative homogeneity of items in burials, and attribute those unique goods as indicative of achieved status or family accumulation of wealth. Additionally, Haskins and Herrmann (1996:114) note that the lack of definitive social classes at Indian Knoll seems to support the hypothesis that social variation within the site is temporally driven rather than indicative of higher social stratification.

According to Hensley (1994:239, 254), burials at Indian Knoll are more fitting of a long-term seasonal site, in accordance with Hofman's (1986) hypothesis. As noted by Hensley (1994:27), aggregation sites serve as a rendezvous location for groups that are dispersed for parts of the year. An aggregate group pattern at Indian Knoll was discerned by Hofman (1986:181), in which most individuals interred were between the ages of 20 to 39 years. According to Hofman (1986:181), a shelter site would see a different distribution of burials in which most individuals were very young or very old, with few burials of individuals between the ages of 20-39 years. Rolingson (1967:395) points out that the presence of non-shell midden sites in close proximity to the shell midden of Indian Knoll might support the hypothesis that the site was utilized seasonally.

Another theory of site use considers the Green River sites to be indicative of a riverine culture where different sites were utilized for specific activities. Winters (1969:108-137) analysis of the Riverton culture was based on the hypothesis that sites had different functional characteristics based on the proportion and types of artifacts between them. In an introduction to the reprint of Webb's (1946) report, Winters (Webb 1974:xvi) compiled and graphed the data originally presented by Webb (1946) and applied his model to the Green River region. In his analysis, Winters (Webb 1974:xvi) suggested that an "Indian Knoll culture" inhabited sites along the Green River archaeological sites for different purposes. In this system, Winters (Webb 1974:xvi) hypothesized Indian Knoll, Barret, and Carlston Annis to be base camps, while Read, Chiggerville, and Ward were thought to be settlements, and Kirkland a hunting camp.

This explanation lends to the hypothesis that Indian Knoll was a seasonal site utilized for the harvesting of shellfish, and fits in accordance with Hofman's (1986) idea of an aggregate site. However, the system has been met with criticism for its lack of strong evidence to support the existence of permanent settlements at Chiggerville, Read, and Ward, and is not accepted as a viable hypothesis in the present day (Marquardt and Watson 2005:634).

Robbins (1977:15) participated in the flotation study of soil samples from Carlston Annis with Watson (1976) and stated that the samples indicated an "intense seasonal occupation." The most common samples obtained from the flotation analysis were the bones of small fish, rodents, turtles, and hickory nuts (Robbins 1977:16). The presence of nut remains coincides with Hensley's (1994) study of several Green River habitation sites, which will be discussed in greater detail in a discussion of diet at Indian Knoll.

Recent efforts have been made to better understand the position of the Green River sites on the landscape using geoarchaeological and zooarchaeological data (Morey *et al.* 2002). The analysis of shell taxa from Indian Knoll revealed that shellfish from the archaeological context of Indian Knoll would have come from a portion of the river with moderately deep water and a relatively quick current, thus suggesting that the main river channel was the choice location for the harvesting of shellfish by the inhabitants of Indian Knoll (Morey *et al.*2002:546-548). Morey *et al.* (2002:548-549) note that the inhabitants of Indian Knoll occupied the site at least in part due to its strategic access to these shellfish resources, but argue that the site was likely was just as important for its physical location as it was for its cultural meaning to the inhabitants of Indian Knoll (Crothers 1999:250; Morey *et al.* 2002:528-529).

Crothers (1999:246-247) postulated the importance of stress on shaping the social organization, mobility, and utilization of resources by the Green River hunter-gatherer groups. This hypothesis puts forward the importance of kinship in maintaining rights to the abundant resources the Green River region had to offer: game, shellfish, and a variety of wild plants and nuts (Crothers 1999:247-249). Additionally, Crothers (1999:249-250) argued that those inhabiting the Green Rivers sites were not socially isolated. Mobility and the potential of trade networks of the Green River inhabitants were also discussed by Winters (1968:175-220).

With regard to social interactions, research on biodistance at Indian Knoll and other Green River and shell midden sites has shed additional light on the movement of groups in the Green River region. A study of cranial metrics by Herrmann (2002:56) demonstrated that there was little morphological difference at Indian Knoll between layers of the site (Herrmann 2002:140, 148), but that Indian Knoll was biologically distant from the Eva site in Tennessee (Herrmann 2002:140). Biodistance between the Green Rivers sites was consistent with a pattern of geographic isolation (Herrmann 2002:173). A non-metric comparative analysis of Green River sites, however, yielded differences between Chiggerville and the Green River sites (Herrmann 2002:145, 171). Herrmann (2002:172) postulated that the differences between the sexes at Chiggerville may be evidence of a patrilocal society with higher mobility among females.

*Diet at Indian Knoll and Green River Sites*

A bioarchaeological study of the skeletal remains at Indian Knoll (Cassidy 1972:137-139) revealed a significant number of growth arrest lines in subadults, suggesting that recurring nutritional deprivations took place in the population. This finding may further contribute to the hypothesis that Indian Knoll was a seasonal site and, in part, utilized for its proximity to seasonal food resources. Given that a number of shell midden sites exist across the southeastern United States, including Indian Knoll, and the general consensus that shellfish were harvested in part for subsistence, one must consider the dietary contribution of shellfish within the Archaic diet.

Freshwater mussels were primarily used as a source of food prehistorically (Klippel, Celmer, and Purdue 1978:257; Parmalee and Klippel 1974:421). In order to determine the dietary contribution mussels may have provided to the Green River inhabitants, Parmalee and Klippel (1974) analyzed the nutritional composition of several species. Mussels were analyzed and found to contain between eight and nine percent protein (Parmalee and Klippel 1974:432), with 100 grams of mussels containing 320 calories (Parmalee and Klippel 1974:431). The comparative analysis of the nutritional composition of both shellfish and other foodstuffs recovered from archaeological contexts of eastern North America indicated that mussels contain fewer calories per unit than other meat resources that may have been available in the Archaic, leading the authors to postulate that they were used as supplementary nutrition (Parmalee and Klippel 1974:432). While other sources of protein analyzed contained far more protein, fat, and calories than shellfish, shellfish were more abundant in carbohydrates and fiber (Parmalee and Klippel 1974:431). This may lend itself to the hypothesis that some Archaic riverine sites are thought to have been seasonally occupied for their mussel resources (Parmalee and Klippel 1974:432). However, the proportion of shellfish required to sustain a population would be considerable (Parmalee and Klippel 1974:433). Similarly, Hensley also agreed that shell remains at Indian Knoll are too few to suggest that mussels were a main source of food (1994:247). Rolingson (1967:395) noted that the variety of remains found in the archaeological context of Indian Knoll, including fishhooks, deer bone, and nuts, may indicate diverse food procurement strategies.

To better understand the formation and utilization of Green River sites, Hensley (1994) sampled a number of shell-free Green River Archaic sites to gain a better understanding of why people inhabited them. Hensley analyzed three sites: Dr. Wan's Floodplain (15Bt45), York Render (15Bt92), and Dr. Wan's Hilltop (15Bt46). Hensley (1994:254) notes that archaeobotanical data indicated a spring and fall occupation, at minimum. According to Hensley (1994:254), there is little evidence that agriculture or horticulture was a primary source of subsistence. Minor amounts of squash (*Cucurbita pepo*) have been found at sites across the region (Hensley 1994:251), which may indicate that minimal horticulture took place (Hensley 1994:75). However, it has been postulated that these may have been used as containers rather than cultivated for consumption (Hensley 1994:72). Nuts were a common finding across the sites excavated by Hensley (1994:140, 195), with hickory (*Carya* sp.), acorn (*Quercus* sp.), and walnut (*Juglans nigra*) found at two of the sites, in addition to a variety of other botanical remains.

The majority of plant remains recovered in a flotation study of Carlston Annis included fruits such as honey locust (*Gleditsia triacanthos* L.), blackberry (*Rubus* sp.), grape (*Vitis* sp.), and persimmon (*Diosporos virginiana* L.), in addition to knotweeds (*Polygonum* sp.), grasses (*Setaria* sp., *Panicum* sp., and *Zizania aquatica* L.), elderberry

(*Sambucus* sp.), and possibly sunflower (*Helianthus annus* L.), in smaller amounts (Crawford 1982:208). Acorn, hickory, and walnut were also a large percentage of remains in the flotation sample, with hickory playing a larger role at Carlston Annis (Crawford 1982:212).

While we do not have a perfect understanding of the people of Indian Knoll and other Green River sites, ongoing research continues to fill in the blanks. Though a lot of archaeological research has been conducted on the Indian Knoll and other Green River Archaic sites, there is still a great deal to learn about the people who in inhabited the sites, why, how, and for how long. The analysis of osteological markers has been the focus of many an Indian Knoll researcher. Over the years, numerous analyses have noted the presence of infectious diseases, in addition to metabolic and hematological disorders, at Indian Knoll and other Green River sites. In order to lay the framework for the present study, a discussion of the etiology of lesions noted in the Indian Knoll skeletal population will follow, preceded by a discussion on the prevalence of these lesions as noted by prior researchers.

# OSTEOLOGICAL MARKERS OF INFLAMMATION & PHYSIOLOGICAL STRESS

Lesions on skeletal remains can be indicative of inflammation, and potentially infection, as well as a host of stress markers indicative of deficiency and metabolic disorders. Inflammatory lesions throughout the body can sometimes be indicative of a single systemic condition, such as syphilis or leprosy, or multiple unrelated conditions, hence the impetus behind the present study. When generalizing the relative health of individuals based on the evidence we have left in bones, we risk overestimating the potential for mortality. Having one instance of inflammation, unless severe, may not be indicative of poor health, but rather that one has a competent immune system capable of producing a reaction. This is the premise of the osteological paradox postulated by Wood *et al.* (1992). Lesions on the bone indicate that an individual had an inflammatory process at some point in their lives. Distinguishing between active, healing, and healed lesions is a crucial step in estimating an individual's state of health at time of death (Wood *et al.* 1992:352). Having an active lesion versus one that is healed or healing is quite different in terms of detecting immune competence (Wood *et al.* 1992:352). Instead of suggesting frailty, healed and healing lesions indicate that a person's immune system was functioning as it should and reacting to the stimuli (Wood *et al.* 1992:352-353), whereas active lesions may indicate a compromised immune system since death soon followed a stimulus (Wood *et al.* 1992:352-353).

As mentioned previously, the purpose of the present study was to detect associations between inflammatory lesions in a sample from the Indian Knoll population. Doing so will open up a discussion of immune response when multiple inflammations are present in an individual. A discussion of the lesions that were analyzed in the present study immediately follows.

*Abscesses*

Bacteria can enter the soft tissues through cracked teeth, pulp exposure, and tooth caries (Hillson 2005:303). A release of fluid by the capillaries produces inflammation at the site of the lesion, initiating the process of flushing the antigens and bacteria (Hillson 2005:304). Abscesses (Figure 1) are suppurative lesions, which is made up of lymphocytes and phagocytes, and bacteria (Hillson 2005:304). The buildup of fluid inside the pulp chamber can cause the pulp to die, leaving an open space (Hillson 2005:304). Pus will escape through the surrounding alveolar bone, and if the body is successful in eradicating the source of inflammation, the surrounding bone will remodel with no loss (Hillson 2005:304).

Lateral abscesses are inflammatory lesions that occur on the side of the tooth root; these are suppurative lesions that do not impact the root directly (Hillson 2005:307). Inflammation may be in response to trauma or a disturbance to the gingiva (Hillson 2005:307). Pus generally escapes through the pocket created from the detachment of the periodontal ligament, but occasionally does so through the gingiva (Hillson 2005:307), and may produce bone loss (Hillson 2005:313).

Additionally, abscesses may form in the alveolar bone surrounding the apical foramen of a tooth. The primary source of blood to the pulp is through the apical foramen


Figure 1. A large abscess affecting the neck and roots of tooth 19 of the mandible. Photo by the author, used with permission.

at the tip of the root (Hillson 2005:307). Abscesses of the apex can involve the accumulation of pus in the socket, and in most cases a bulge and inflammation of the periodontal tissue surrounding the root apex (Hillson 2005:308). Bone loss is uncommon in acute periapical abscesses since fluid usually escapes through the bone, though it does occur (Hillson 2005:308-309). Periapical periodontitis is caused by an inflammation of the pulp during which the products of inflammation exit from the apical foramen (Hillson 2005:307). The surrounding periodontal tissues become inflamed, and bone remodeling may take place to make way for granulation tissue (Hillson 1996:285). Bone loss typically occurs in cases of periapical periodontitis where a granuloma is present (Hillson 2005:308). Acute periapical abscesses commonly form from periapical granulomas (Hillson 1996:285). Periapical abscesses may become chronic as pus slowly accumulates

(Hillson 2005:309), which may escape through the open root canal or alveolar bone and surrounding mucosa (Hillson 2005:309).

*Auditory Exostoses*

Exostoses are characterized by benign bony growths at the external auditory meatus or in the auditory canal (Aufderheide and Rodríguez-Martín 1998:254), though the etiology of these bony growths are a source of debate. Early works described these growths involving the external auditory meatus as a benign neoplasm or osteoma, and more recently the term "exostosis" has been used to describe the condition, according to Aufderheide and Rodríguez-Martín (1998:254). However, it has also been argued that there are differences between osteomata and exostoses related to how and where bone production occurs. Osteomata (Figure 2) are bony growths that are generally unilateral, and can be attached to bone or pedunculated (DiBartolomeo 1979:8; Kennedy 1986:403; Sheehy 1958:1667), meaning they are attached with a stalk of tissue. Exostoses (Figure 3) are generally and broad (Sheehy 1958:1667). The most widely accepted hypothesis in the literature is that an irritation of the periosteum of the external auditory canal, by cool wind and water temperature, results in the formation of exostoses.

Exostoses have been attributed to a genetic predisposition with an external stimulant (DiBartolomeo 1979), as well as an exclusively environmental, or incidental, cause (Hong 1965; Kennedy 1986; Kuzminsky *et al.* 2016; Okumura *et al.* 2007; Standen *et al.* 1997). Race was proposed to play a role in the predisposition of certain individuals to develop the condition (DiBartolomeo 1979:15-16). However, evidence is continually building to support the hypothesis that cold water and air chill may induce the formation of exostoses.

Exostoses are virtually absent from skeletal remains under the age of 20 years which further supports an environmental etiology (Aufderheide and Rodríguez-Martín 1998:255). DiBartolomeo (1979) found that many of his patients had them, and yet only 30.0 percent claimed to have a memory of mild ear infections in their childhoods (DiBartolomeo 1979:10). However, DiBartolomeo (1979:9) did note that many of his patients described engaging in activities involving the ocean frequently as adolescents. Kennedy tested a hypothesis developed by Van Gilse (1938), relating the trauma of cold water to these boney growths of the ear. To test this hypothesis, Kennedy (1986:404) looked at the frequencies of exostoses in both published and unpublished works from different geographic regions representing different latitudes. The hypothesis tested surmised that exostoses would not be found in polar and subpolar regions because the water would be too cold to realistically enter on a regular basis, and they would also not be found in tropical latitudes where the water is warmer (Kennedy 1986:407). Exostoses were found to be low in frequency in portions of Europe, Asia, and the Pacific Northwest where latitudes extended beyond 45 degrees latitude north (Kennedy 1986:408, 411), as well as in the 0-30 degree latitudes of Hawaii, Easter Island, and Polynesia. Populations that lived in the ideal 30-45 degree latitude range were more likely to have exostoses, as would be expected; regions in this latitudinal range include the American Southwest, Near East, and parts of Australia (Kennedy 1986:408). A study by Okumura *et al.* (2007) supports this finding. Okumura *et al.* (2007:564) formulated that air temperature played a more significant role in the formation of exostoses, while water temperature was a contributing factor, supported by the increased occurrence of exostoses in groups where wind chill was increased.


Figure 2. Possible osteoma, approximately 2/3 of the auditory meatus is occluded; condition is unilateral in the individual pictured. Photo by the author, used with permission.

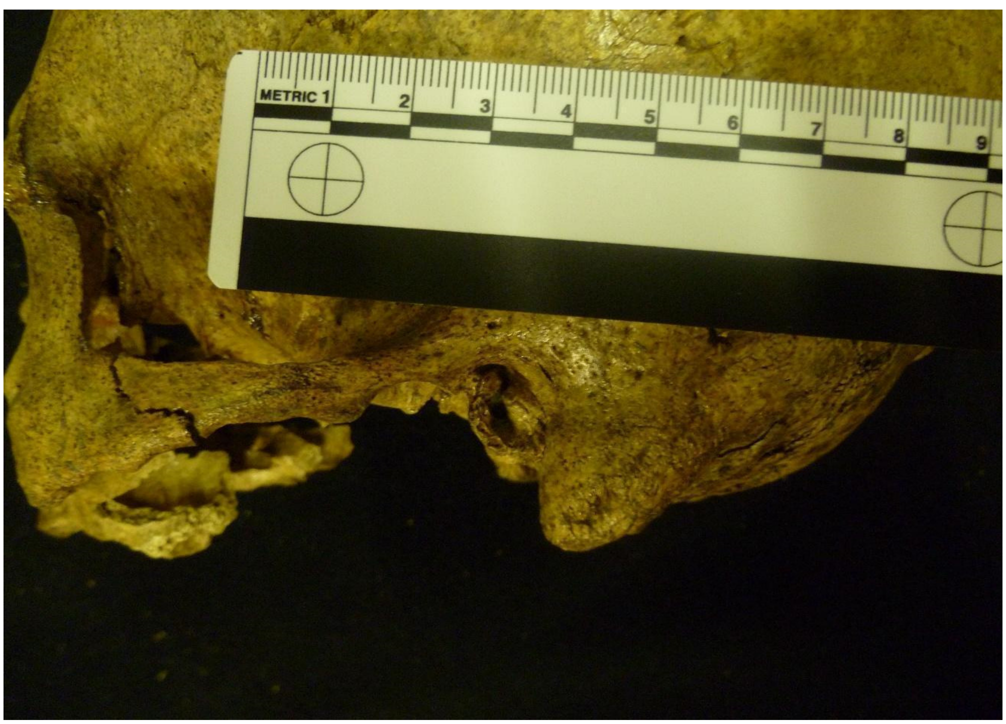


Figure 3. Likely exostosis of the left auditory meatus; condition is bilateral in the individual pictured (right side not shown). Photo by the author, used with permission.

Sex differences were evident in Kennedy's (1986) literature review. However, it is also thought that gender roles correlate to cold water and wind exposure, thereby influencing the occurrence of exostoses between the sexes (Okumura *et al.* 2007:546). Though data were not available, Kennedy (1986:409) noted that Japanese pearl divers are known to exhibit ear exostoses. Similarly, female divers in Korea were found to have exostoses in 31.0 percent of cases, whereas women who did not dive were absent of the condition (Hong 1965:108). DiBartolomeo (1979:9) did note that his patients were all Caucasian, ranging from age 13-71 years, and the majority of those exhibiting exostoses were males between the ages of 25 and 35. A study by Standen *et al.* (1997:125) also found that exostoses were most common in males, and proposed an environmental cause of the lesions. However, a study by Okumura *et al.* (2007:563) did not find a significant difference in the proportion of exostoses between the sexes.

The condition has been found in Neolithic bones from Japan dating to around 5000 B.C.E. (Aufderheide and Rodríguez-Martín 1998:255). Additional evidence for exostoses has been found in Neanderthals; the skeleton known as Shanidar 1 exhibited what Trinkhaus (1983:98) referred to as "tubercles" (1983:69), however Shanidar 2 did not. Many prehistoric coastal populations exhibit the condition (e.g. Kuzminsky *et al.* 2016; Okumura *et al.* 2007).

*Cribra Orbitalia and Porotic Hyperostosis*

Cribra orbitalia and porotic hyperostosis are descriptive terms relative to a morphological condition and its location on the human cranium (Schultz 2001:106-107). For the purposes of the present study, orbital porosity is synonymous with cribra orbitalia, just as cranial porosity is synonymous with porotic hyperostosis.

Commonly thought to result from anemia, cribra orbitalia is characterized by a hair-on end appearance (Aufderheide and Rodríguez-Martín 1998:350; Roberts and Manchester 2005:229), porosity (Figure 4), or thickening of the diploë in the superior wall of the eye orbits (Roberts and Manchester 2005:229). Ninety percent of cases will exhibit bilateral lesions (Aufderheide and Rodríguez-Martín 1998:349). The manifestations of cribra orbitalia and porotic hyperostosis are categorized by severity much like any lesion, and can involve orbital lesions alone, or both orbital and calvarium lesions (Roberts and Manchester 2005:230).

Porotic hyperostosis is thought to be the manifestation of iron-deficiency that manifests on the calvarium. In the early stage of porotic hyperostosis, fine porosity can be seen on the calvarium (Figure 5), which may expand into larger pits and coalesce (Schultz 2003:103). Hair-on end bone production (Aufderheide and Rodríguez-Martín 1998:350) may occur, in addition to a slight thickening of the vault; it has been stated that the latter is difficult to observe without a cross-section of bone (Schultz 2003:103).

Cribra orbitalia and porotic hyperostosis can be found in infancy, but also occurs throughout adolescence (Aufderheide and Rodríguez-Martín 1998:349). Cribra orbitalia is more commonly found in populations than porotic hyperostosis, and has been suggested to be a better indicator of nutritional stress (Aufderheide and Rodríguez-Martín 1998:350; Roberts and Manchester 2005:230).

Attributing cribra orbitalia and porotic hyperostosis to an iron-deficient cause is a source of contention in the scholarly community (Waldron 2009:136). Iron-deficiency can result from the loss of blood, malabsorption of iron, or a diet insufficient in iron (Walker *et al.* 2009:111). Iron is needed to form hemoglobin in new red blood cells

(Roberts and Manchester 2005:226). The normal life span of red blood cells is 120 days (Roberts and Manchester 2005:226; Walker *et al.* 2009:111), but red blood cells in anemic individuals last half as long (Roberts and Manchester 2005:226). While iron-deficient anemia is the most common variation of anemia in the present day, two genetic forms of anemia also occur (Roberts and Manchester 2005:232-233): sickle-cell anemia, and thalassemia (Roberts and Manchester 2005:232-233; Walker *et al.* 2009:111). Hemolytic anemia leads to an expansion of the marrow, which produces porosity on the external vault (Waldron 2009:136; Walker *et al.* 2009:111).

It has been argued, however, that porotic hyperostosis and cribra orbitalia cannot be explained by iron-deficiency anemia because the life of red blood cells is much too shortened to produce marrow expansion (Waldron 2009:137; Walker *et al.* 2009:112). The reasoning behind this hypothesis is that iron is required for the production of red blood cells, and iron reserves are not present in patients with clinical iron-deficient anemia; therefore, marrow hypertrophy simply cannot occur in a state of iron-deficiency (Walker *et al.* 2009:112). Instead, Walker *et al.* (2009:112) suggest that megaloblastic and hemolytic anemia provide a much better explanation for cranial and orbital porosity. Hemolytic anemia is hereditary, and includes both sickle cell anemia and thalassemia (Walker *et al.* 2009:112). Megaloblastic anemia has a root cause in nutritional deficiencies and malabsorption in New World populations, and can also cause hypertrophy (Walker *et al.* 2009:114-115).

Active lesions associated with porotic hyperostosis and cribra orbitalia are nearly always associated with subadults (Walker *et al.* 2009:111). In some cases, both conditions occur in the same individual, but generally cribra is a more likely finding in


Figure 4. Macroporosity on the left anterio-lateral portion of the orbit. Photo by the author, used with permission.


Figure 5. Micro and macro porosity on the posterior left parietal with raised bone in between. Photo by the author, used with permission.

skeletal populations (Walker *et al.* 2009:115). It has been postulated that cribra may the less severe manifestation of anemia, perhaps lending to its prevalence over porotic hyperostosis, or that age may play a role in the location of lesions (Walker *et al.* 2009:115).

Roberts and Manchester (2005:234) warn that it can difficult, if not impossible, to discern the specific anemia suffered by an individual based solely on skeletal evidence because the cranial and orbital lesions are too similar between all forms of anemia. Similarly, lesions associated with anemia can be difficult to diagnose due to their similarity with other skeletal lesions; scurvy and rickets, for example, can also produce similar lesions on the cranium (Roberts and Manchester 2005:230; Schultz 2003:102). It is precisely for this reason that microscopic, versus macroscopic, analysis is the preferred method in determining the etiology of orbital and cranial lesions (Schultz 2001; Wapler *et al.* 2004). It has been demonstrated that diagnosis of lesions may change when analyzed macroscopically and microscopically (Schultz 2001:109). Possible causes of cribra orbitalia are not limited to anemia, but also include rickets, osteitis, hyper-vascularization, and postmortem erosion (Wapler *et al.* 2004:335). Porosity on the cranium can have just as many possible etiologies, including metabolic and hematological conditions such as anemia, rickets, and scurvy, in addition to osteomyelitis, periostitis, and postmortem damage (Schultz 2001:121).

Additionally, Mann and Hunt (2005:22) make the distinction that porotic hyperostosis is characterized by both porosity and bone thickening. It should also be mentioned that anemia is a condition that can be aggravated by the pre-existence of infection (Roberts and Manchester 2005:228). Similarly, patterns of porosity in individuals may differ between populations and should not be dismissed as a normal variation, according to Roberts and Manchester (2005:230). Similarly, aging adults can exhibit patterns of pinpoint porosity (Mann and Hunt 2005: 25) on the elements of the external vault.

*Enamel Hypoplasia*

Linear enamel hypoplasia is a condition that is characterized by a defect in the enamel of the tooth resulting from nutritional deficiency (Langsjoen 1998:405), such as an insufficient diet or malabsorption of nutrients. Enamel hypoplasia is commonly caused by an arrest in growth resulting from inadequate consumption of vitamins and minerals (Langsjoen 1998:405; Hillson 2005:174-176; Ortner 2003:595). Such stress creates a "lesion" on the surface of the tooth that can take a number of forms, such as horizontal or vertical grooves (Figure 6), pits, or boundary opacity (Hillson 2005:169-170). Linear enamel hypoplasia is most often seen on the front and lateral incisors, and as well as the canines (Hillson 1996:167).

Enamel defects are indicative of non-fatal stressors on the human body, a condition during which the body reroutes the resources it is taking in to the most important functions for survival (Langsjoen 1998:405). Enamel defects are produced by an interruption in the production of enamel (Langsjoen 1998:406; Buikstra and Ubelaker 1994:56; Hillson 2005:168). Amelogenesis is the production of dental enamel, and the process is normally incremental, whereby matrix is produced by ameloblasts from dentin to crown, after which they become mineralized (Langsjoen 1998:406; Hillson 2005:155). The maturation phase is characterized by the removal of protein and water (Hillson 2005:155). A delay in the secretion of the matrix is what contributes to linear

Figure 6. Horizontal enamel hypoplasia on the central incisors of the maxilla. Photo by the author, used with permission.

enamel lesions, and a delay in the maturation phase contributes to hypocalcifications (Hillson 2005:168). Resumption of matrix mineralization indicates absence of the stressor that was previously prohibiting the body to form enamel (Langsjoen 1998:406). However, the previous layer of enamel that never mineralized does not resume, and instead a new layer of enamel begins to form and mineralize (Langsjoen 1998:406). This event produces a hypoplastic groove, a condition in which the halt in mineralization produces a layer of enamel that is very thin when compared with normal enamel (Langsjoen 1998:406). Enamel cannot heal or remodel itself, thus explaining why hypoplastic lesions are permanent indicators of nutritional deficiencies (Langsjoen 1998:406-407). While enamel defects can be caused by systemic stress, isolated incidences of hypoplasia can be indicative of trauma when occurring on one or two teeth (Buikstra and Ubelaker 1994:56; Hillson 1996:165, 2005:315).

*Periodontal Disease*

Also known as periodontitis in its severe manifestation, the condition is defined as a chronic inflammation of the periodontium that results in the destruction of underlying bone. When chronic inflammation of the gingiva occurs, this interferes with the collagen fibers forming the periodontal ligament, thus producing the bone loss (Figures 7 and 8) seen in periodontitis (Hillson 2005:304).

Inflammation is caused by gram-negative bacteria that make up a biofilm that covers the surface of the tooth between the root and junctional epithelium (D'Aiuto *et al.* 2004:159). The periodontium is the tissue surrounding the tooth that supports the teeth in the mandible and maxilla. When inflammation occurs in the periodontium, it can be characterized by localized or general alveolar bone loss in skeletal samples (Langsjoen 1998:402; Hillson 2005:305). In present-day populations, periodontal disease affects around 75.0 percent of adults and does not discriminate between the sexes; similarly, the frequency of occurrence increases as a person ages (Langsjoen 1998:401). Periodontal disease is the leading cause of tooth loss in living individuals (Hillson 2005:306), and tends to increase in prevalence with aging (Langsjoen 1998:401; Hillson 2005:305).

Inflammation that affects the gingiva only is called gingivitis (Hillson 2005:305; Ortner 2003:594), while chronic inflammation that impacts the periodontal tissues is referred to as periodontitis (Hillson 2005:305; Ortner 2003:593). While the localized gingivitis is sometimes preceded by a traumatic injury to the mouth, more often it is the result of bacteria (Hillson 2005:305). Inflammation can occur in response to trauma or irritation of the gingiva in the mouth during which it can begin to come away from the tooth, a condition called a periodontal pocket (Hillson 1996:261).

Figure 7. A relatively healthy mandible with no evidence of alveolar resorption below the molars. Photo by the author, used with permission.

Further disruption of the collagen fibers in the gingiva and periodontal ligament leads to the progression of inflammation (Hillson 1996:262). The periodontium will begin recede from the cemento-enamel junction (CEJ) and expose the cementum of the root (Langsjoen 1998:402; Hillson 1996:262; Ortner 2003:593). Impacts to the tissue create defects in the underlying bone which can be either vertical or angular at the root (Langsjoen 1998:402). Alveolar bone loss tends to be symmetrical and most commonly affects the molars (Hillson 1996:265), and condition associates with aging in adults (Hillson 2005:306). Periodontal disease is also characterized by alveolar bone porosity (Hillson 2005:312).

Dental plaque contains bacteria, and over time is deposited at the cervical root of teeth in the form of calculus (Langsjoen 1998:401; Ortner 2003:593). The fibers of the periodontal ligament are impacted by the stagnant bacteria as it finds its way into the space. Additionally, it is thought that inflammation can be induced by the presence of plaque and calculus alone (Ortner 2003:593). In antiquity, periodontal disease was most commonly preceded by severe attrition (Langsjoen 1998:401) and can be accelerated by a diet heavy in gritty foods (Langsjoen 1998:402). When the teeth are extensively worn down beyond their crowns and the teeth no longer touch, the teeth no longer form a mesial barrier to prevent food from entering between them (Langsjoen 1998:401). Thus, when food is forced into this space during the chewing process, bacteria lingers (Langsjoen 1998:401).

Tooth loss occurs when too much bone has been lost, and remodeling of the bone takes place (Larsen 2015:81). Tooth loss and periodontal disease increased in prevalence with the shift from foraging to agriculture (Larsen 2015:81). In pre-agricultural societies, tooth loss most likely resulted from pulp exposure from attrition (Larsen 2015:81). Tooth loss is most likely to occur on the posterior maxillae and mandibles, often affecting the molars (Larsen 2015:81). Archaeological samples indicate that sex did not play a role in the prevalence of tooth loss, but recent evidence suggests the risk of periodontal disease is greater in males (Larsen 2015:83).

Similarly, poor collagen production can interfere with the periodontium. Alveolar resorption may also be the result of scurvy, though it may not be a diagnostic feature of the condition specifically according to Maat (1982:90). However, Maat (1982:87) identified alveolar resorption in as many individuals with a vitamin C deficiency as those with no signs of scurvy.

Figure 8. Slight resorption and porosity of the alveolar bone below the mandibular molars. As seen in an individual from the Indian Knoll population. Photo by the author, used with permission.

*Supraeruption of Teeth*

It has been assumed and generally accepted that a distance of 2 mm or greater between the cemento-enamel junction (CEJ) and alveolar crest is evidence for the presence of periodontal disease (Clarke and Hirsch 1991:241), with a distance of 3 mm or greater reflecting aggressive periodontal disease (Hillson 2005:312). It has been suggested in the past that measuring the cemento-enamel junction can present researchers with a snapshot of an individual’s health throughout their life (Clarke 1993:1). However, this assumption is based on the concept that the increased distance between the cemento-enamel junction and alveolar crest is indicative of bone loss (Clarke 1993:1). Bone loss with age is not supported, but we do know that the teeth continue to erupt throughout one’s lifetime (Clarke 1993:1; Clarke and Hirsch 1991:243; Hillson 1996:263; Whittaker *et al.*1985:493), thus making it possible to score for alveolar resorption in error.

Increased distance between the CEJ and alveolar crest result from two processes: continuous eruption to maintain lower facial height that is lost from tooth wear, and coronal growth from the cranial sutures to allow maxillary teeth to maintain articulation with mandibular teeth (Clarke and Hirsch 1991:241). Clarke (1993:3) recommends that a correction of 0.1 mm per year be done to account for the continuous eruption of teeth, and exclude false positive samples when testing for periodontal disease. This amount of supraeruption is significant, especially when looking at individuals of middle or old age in studies involving periodontal disease.

Additionally, there are two infectious causes of periodontal attachment loss: periodontal disease (gingivitis or periodontitis), and abscessing (Clarke and Hirsch 1991:241), as discussed previously. Of particular importance in the matter of diagnosing alveolar bone loss is making this distinction, and incorporating the possibility of supraeruption. Formerly, an overestimation of periodontitis resulted from a diagnosis that included bone loss from lateral abscesses (Clarke and Hirsch 1991:241).

*Periosteal New Bone Formation*

Periosteal lesions are one example of an inflammatory response that can be caused by infection, but infection is not the only cause of lesions (Weston 2012:493). Periosteal lesions are characterized by involvement of the periosteum, which is a thin layer of tissue that covers bone and it responds to stimuli by forming new bone (Waldron 2009:20), thus healing the bone damage that results from either infection, stress, or trauma (Waldron 2009:115; Weston 2012:493). Healing is characterized by absorption of dead tissues by macrophages and osteoblasts, and periosteal new bone is laid down as woven bone, and ultimately forms lamellar bone through the healing process (Weston 2012:494). Activity of the periosteal new bone formation can be determined by looking at the edges of the lesion, color, and texture – active lesions are sharp, porous, loosely attached, and lighter in color, whereas healing lesions begin to smooth and blend into the surrounding bone (Mann and Hunt 2005:183). Mann and Hunt (2005:183) describe active periostitis as “small, loosely attached sheets of tree bark or thin layers of sponge.” Systemic infectious diseases such as tuberculosis, leprosy, treponemal disease, and polio, as well as localized infections like sinusitis, can cause lesions in the bone if they are not treated (Aufderheide and Rodríguez-Martín 1998; Waldron 2009). The most common infectious cause of periosteal new bone formation is the pathogen *Staphylococcus aureus*, though a number of pathogens can cause bone lesions (Waldron 2009:84-85). Osteomyelitis is caused by *Staphylococcus* in 90 percent of cases where it occurs, followed closely by *Streptococcus* as the second most likely cause (Ortner 2003:181). A number of conditions, other than infection, can produce periosteal new bone formation.

*Inflammation vs. Infection.* The term “periostitis” has been a source of contention as of late due to its widespread use in anthropological literature as a term to describe the deposition of new bone (Weston 2012:492-493). Periostitis refers specifically to an inflammation of the periosteum, the thin layer of tissue that adheres to bones. Use of this term implies two things: inflammation, and the involvement of the periosteum, not the bone itself (Weston 2012:493). Hence, this term may not best fit all scenarios where new bone is forming (Weston 2012:493). Several terms have been proposed for use to generalize new bone formation, such as “periosteal new bone formation” (Ragsdale 1993:465).

One problem in the analysis of skeletal remains is that new bone formation has frequently been attributed to “non-specific infection,” and yet inflammation is not synonymous with infection (Weston 2012:493). Infection is caused by a pathogen and the bone reacts with an inflammatory response to kill the pathogen and repair damage done by it (Weston 2012:493). Inflammation indicates the response on part of the body to a number of stimuli (Weston 2008:49), both infectious and non-infectious (Weston 2012:493). The vague term “non-specific infection” indicates a lack of knowledge about the specific cause of new bone formation (Weston 2012:503). Larsen (2015:88) suggests that the prevalence of periosteal lesions through time, and among groups with varying mobility, may potentially be evidence of the nature of the lesions observed. That is, in later prehistoric populations with a higher concentration of individuals, and decreasing mobility, periosteal lesions may be due to infection as opposed to trauma (Larsen 2015:88).

Attempting to diagnose the specificity of an infection is practical when one is trying to make generalizations about a population in regard to that disease. However, one might argue that broader implications can be made when diagnosing infection more generally, always being careful to eliminate traumatic lesions. Weston’s advice is especially important in this study since efforts to detect the correlation between infection and nutritional deficiencies were undertaken; further discussion on the importance of this measure and steps taken to ensure testability will be described in detail later. Despite the debate surrounding the use of the term “non-specific infection,” this study did not attempt to diagnose the specific etiology of periosteal new bone formation in the hopes of making broader associations between simultaneous infections, and correlations between infection and nutritional deficiencies.

*Categorizing Periosteal New Bone Production.* The tibia is a common location of periosteal lesions, but the exact reason is unknown (Larsen 2015:88; Roberts and Manchester 1995:129). The tibia has little anterior protection from trauma (Larsen 2015:88), which may explain why it is frequently the site of lesions. Similarly, it is possible that chronic or severe infections of the skin can eventually infect the bone (Roberts and Manchester 1995:129).

Periosteal new bone formation has traditionally been categorized into three stages of severity: periostitis, osteitis, and osteomyelitis, depending on whether the infection involves only the periosteum, compact bone, or both, respectively (Roberts and Manchester 1995:126). Osteomyelitis, the most severe manifestation of bone lesions and includes involvement of the medullary cavity, and often bone-eroding abscesses resulting from an infectious agent (Roberts and Manchester 1995:126).

Researchers (Edeiken *et al.* 1966; Ragsdale 1993; Ragsdale *et al.* 1981) have differentiated categories of new bone formation and possible etiologies, while others (Strothers and Metress 1975) have devised a system for identifying the processes of periostitis and osteomyelitis. The categorizations in Strothers and Metress (1975) were useful in devising the scoring system used in this study, though it did not directly lend much terminology to it. Strothers and Metress (1975) described four stages of periostitis with gradual bone formation that begins as raised striations on the surface of long bones, gradually forming sheet-like formations of new bone as the striae blend together (Strothers and Metress 1975:4, 6).

Edeiken and colleagues (1966:709) also defined a number of types of new bone formation and their possible causes, both infectious and non-infectious. Edeiken and colleagues (1966:709) differentiated between two types of reactions: solid periosteal reactions, and interrupted periosteal reactions. Solid reactions were defined as being greater than 1 mm thick, with “uniform density; that is, the entire sheet of periosteal new bone looks the same” (Edeiken *et al.* 1966:709-710). The authors defined interrupted periosteal reactions as being pleomorphic (Edeiken *et al.* 1966:715), meaning the size and shape of the bone cells varies. Additionally, the authors described interrupted periosteal reactions as fast-progressing and lacking stability, suggesting that they can occur in a matter of days (Edeiken *et al.* 1966:715).

With these systems in mind, an interesting form of periosteal new bone formation has been described in Anglo-Saxon skeletons in England. In this population, inflammatory lesions were characterized by fine pitting, striations, and sequential plaque-formation on the cortical surface (Roberts and Manchester 1995:129-130). Raised striae on the long bones was also noted in the Morris Site (CK39) in Cherokee County, Oklahoma (Brues 1959:67). This manifestation of inflammation is also mentioned in the system described by Strothers and Mettress (1975:4).

While understanding the etiology of new bone formation is useful in performing a differential diagnosis, several problems can be extrapolated from this venture. There is no standard or agreed-upon scoring system that incorporates all aspects of bone formation (Weston 2012:499). Additionally, scoring systems frequently differ depending on the researcher conducting the study, and some scoring systems are not included in bioarchaeological manuscripts, making it difficult to accurately reproduce similar results. While Buikstra and Ubelaker (1994) provide a scoring system for different pathologies and traumatic injuries by element, the present study did not utilize this system. As mentioned previously this study did not attempt to diagnose the specific etiology of skeletal lesions in the hopes of making broader correlations between instances of multiple infectious processes, and correlations between infection and nutritional deficiencies. Periosteal new bone formation can result from a number of different conditions, both infectious and non-infectious, and for this reason a number of potential diagnoses of the skeletal lesions discovered in this sample population will be discussed in greater detail.

*Etiology of Periosteal New Bone Formation*

While periosteal lesions are an example of an infectious process that is capable of inducing new bone production, trauma can also trigger periosteal new bone formation, though it tends to be unilateral in nature depending on the nature. Both of these processes are characterized by inflammation, first and foremost. Infection and metabolic conditions are more likely to cause bilateral lesions due to their systemic nature. Bilateral involvement is relatively common in treponemal disease (Rothschild and Rothschild 1996:559), or metabolic disorders such as scurvy (Aufderheide and Rodríguez-Martín 1998:311, 349; Maat 1982:88), though scurvy is associated with traumatic hematomas.

*Scurvy.* Scurvy results from a Vitamin C deficiency, also known as L-ascorbic acid. Vitamin C is found in a number of cultivated food sources, and is particularly abundant in citrus fruits, broccoli, bell peppers, kiwi, and strawberries (U.S. Department of Agriculture 2011; U.S. Department of Health and Human Services 2016). Ascorbic is a necessary component in the production of collagen, which is present in bone (Tuross 2003:68) and connective tissues (U.S. Department of Health and Human Services 2016), such as the periosteum. When collagen production is interrupted, wounds do not heal as they should (U.S. Department of Health and Human Services 2016), which results in increased hemorrhaging when trauma occurs. A vitamin C deficiency can also stun growth; lack of ascorbic acid results in defective osteoid formation (Aufderheide and Rodríguez-Martín 1998:310; Ortner 2003:383). In adults the walls of blood vessels are weakened, leading to an increased risk of hemorrhaging (Aufderheide and Rodríguez-Martín 1998:310; Ortner 2003:383-384).

Scurvy is characterized by the formation of petechiae and ecchymoses, which are generally bilateral in nature (Aufderheide and Rodríguez-Martín 1998:311; Maat 1982:88). Lesions are most likely to occur on the lower extremities, such as the tibia where trauma can occur more easily (Aufderheide and Rodríguez-Martín 1998:311). Defective collagen production in individuals with scurvy reduces the normally tight fixation of the periosteum to the underlying bone, making the periosteum more likely to sustain hemorrhaging from minor injuries (Aufderheide and Rodríguez-Martín 1998:311). Hematomas are typically moderately sized and can occur on the diaphysis of elements (Aufderheide and Rodríguez-Martín 1998:311), making periosteal new bone formation in scorbutic individuals difficult to discern from traumatic lesions in a healthy individual.

However, cranial lesions are more common than those in the long bones of the body (Ortner *et al.* 2001:344, 2003:390). Cranial lesions are attributed to an increased risk of bleeding due to the utilization of muscles in mastication (Ortner and Erickson

1997:213). On the cranium, scurvy manifestations have been described as resembling the appearance of an orange peel, and may appear shiny (Sinnott 2013:102). In the orbits, Ortner (2003:388-389) describes lesions that resemble the porosity in anemia, but without hyperplasia. Scurvy tends to be more common in subadults than adults (Ortner *et al.* 2001:344, 2003:387). Clinically, scurvy also results in the loss of teeth due to weakened collagen due to trauma induced by chewing (Ortner 2003:387, 593).

Given that scurvy is associated with increased bleeding due to decreased collagen production, iron-deficiency is a related topic. Walker *et al.* (2009) suggest that cribra orbitalia may have a more complex root cause than porotic hyperostosis, linking anemia to sub-periosteal bleeding resulting from scurvy (2009:110).

*Treponemal Disease.* Three species of the bacterium *Treponema* can produce skeletal lesions: syphilis in both venereal and congenital varieties (*Treponema pallidum pallidum*), yaws (*Treponema pertenue*), and bejel (*Treponema pallidum endemicum*) (Aufderheide and Rodríguez-Martín 1998:154). Each variation of treponemal disease is associated with different geographic locales (Aufderheide and Rodríguez-Martín 1998:155); yaws and primarily found in tropical and sub-tropical regions, while bejel is most likely to occur in rural, sub-tropical regions with low humidity, and syphilis is not limited to a specific geographic region (Aufderheide and Rodríguez-Martín 1998:154), demonstrating that overlap can occur. Skeletal involvement is similar between all three manifestations of the *Treponema* bacterium, with the tibia being the most commonly affected element (Aufderheide and Rodríguez-Martín 1998:156-158).

Syphilis is characterized by venereal and congenital varieties. Congenital syphilis occurs in children who acquire the disease from an infected mother *in utero* (Aufderheide and Rodríguez-Martín 1998:164). Congenital syphilis manifests in two specific lesions that adults who acquire the disease never get: Hutchinson's incisors, and mulberry molar (Aufderheide and Rodríguez-Martín 1998:166). Venereal syphilis is characterized by both gummatous and non-gummatous lesions (Aufderheide and Rodríguez-Martín 1998:158; Ortner 2003:285-286; Waldron 2009:105). Non-gummatous lesions are characterized the presence of periostitis, osteitis, and osteoperiostitis (Aufderheide and Rodríguez-Martín 1998:158; Ortner 2003:285-286), depending on the severity of infection. Hackett (1976:106) proposed that syphilis be diagnosed by the presence of either fine or coarse striae on the long bones accompanied by expansions.

Gummatous lesions can occur on the limbs as well as the cranium; gumma formation on the cranium is referred to as *caries sicca* and is indicative of tertiary syphilis (Aufderheide and Rodríguez-Martín 1998:160-161). Gummatous lesions are characterized by the localized necrosis of bone in the space immediately occupied by the gumma, with sclerotic bone surrounding the depression (Aufderheide and Rodríguez-Martín 1998:160). Coalescing of gummas occurs and results in crater-like depressions on the calvarium, forming a sort of "valley" between the gummatous lesions (Aufderheide and Rodríguez-Martín 1998:161-162). In most cases the outer table and diploë succumb to necrosis, but the inner table is spared; this is not always the case, however, and some syphilitic lesions destroy all layers of bone (Aufderheide and Rodríguez-Martín 1998:162).

Syphilis commonly occurs bilaterally throughout the body, which was evidenced in a study by Rothschild and Rothschild (1996:559). The same bones on which syphilitic lesions manifest are much the same as those in cases of yaws and bejel, and include, in order of prevalence, the tibia, calvarium, nasals, clavicle, vertebrae, in addition to the bones of the leg and arm (Aufderheide and Rodríguez-Martín 1998:158). Although it does produce a wide range of skeletal manifestations not always seen in bejel and yaws, syphilis is the least likely variation of Treponematosis to produce skeletal lesions (Rothschild *et al.* 2000:938). However, the most common site of syphilis, over any other element, is the tibia (Ortner 2003:283). A study by Hackett (1976:27) broke down lesion types and attempted to establish a diagnostic criterion between the treponemal infections; syphilis produced the highest number of lesions in this study. In a study by Hackett (1976:19), tertiary syphilitic lesions were the most commonly noted in skeletal remains, probably due to their severity.

Yaws is a chronic, non-venereal form of treponematosis common in parts of equatorial Africa, the equatorial Americas, southwest Asia, Australia, and the Pacific Islands (Aufderheide and Rodríguez-Martín 1998:155). It is spread by human contact and disproportionately affects the males (Aufderheide and Rodríguez-Martín 1998:155). Skeletal lesions affect the tibia in most cases, but can also be found on the bones of the leg and lower arm, as well as the hands and feet, a condition known as Dactylitis (Aufderheide and Rodríguez-Martín 1998:156). Periostitis is common and results in new bone production, cortical thickening, and in tertiary cases, a condition known as saber shin (Aufderheide and Rodríguez-Martín 1998:156). Saber shin and tibial bowing will be discussed in greater detail in the next section. Cranial lesions may also present and manifest in crater-like depressions of the calvarium, in addition to nasal involvement and perforation of the hard-palate (Aufderheide and Rodríguez-Martín 1998:156). Lesions tend to manifest most often in children because the disease is often contracted by subadults (Ortner 2003:275)

Bejel is commonly found in regions of poor socioeconomic prosperity and warm, arid climates, and manifests in skeletally much the same as in Yaws (Aufderheide and Rodríguez-Martín 1998:157). The tibia is the most likely to be affected by lesions, as well as the nasals (Aufderheide and Rodríguez-Martín 1998:157). Saber shin is the common manifestation of bejel on the tibia (Aufderheide and Rodríguez-Martín 1998:157). Gummatous lesions do occur in cases of bejel in the tertiary stage of infection (Aufderheide and Rodríguez-Martín 1998:157). Skeletal manifestations are seen in one to five percent of cases (Aufderheide and Rodríguez-Martín 1998:157).

Treponemal disease has been the source of debate regarding its origin, mainly whether or not it was present first in the New World or Old World. According to Baker and Armelagos (1988:703), Old World evidence of treponemal disease is rare. Isolated cases have been identified on a few bones with unknown provenience, further complicating the question of Old World origin. Similarly, an examination of 16th century burials at leprosaria in Europe failed to detect any evidence of treponematosis (Aufderheide and Rodríguez-Martín 1998:169), though this is not sufficient evidence alone to say that it did not have an Old World presence.

*Bowing of the Tibiae*

A number of causes can contribute to the anterior bowing of the legs (Figure 9), including treponemal disease and rickets. Rickets can produce tibial bowing anterior and both medial-lateral bowing (Aufderheide and Rodríguez-Martín 1998:307; Waldron 2009:129). Rickets is caused by a deficiency of Vitamin D and results in poor mineralization of the bones, and thus the cellular structure of the bone is different than that of a healthy bone. The process of cartilage calcification in the bones is disrupted in rickets (Aufderheide and Rodríguez-Martín 1998:306). The bone is impacted by unsubstantial vascularization which inhibits the removal of collagen from the forming bone (Aufderheide and Rodríguez-Martín 1998:306). Subsequently, the bowing of femora, tibiae, and fibulae is attributed to the weight of an individual being carried on insufficiently mineralized bones (Aufderheide and Rodríguez-Martín 1998:307). This also explains why fractures in the leg bones are more likely to occur in individuals with rickets (Aufderheide and Rodríguez-Martín 1998:307).

Figure 9. Anterior bowing of the tibiae. Photo by the author, used with permission.

Tibial bowing in rickets is a true bowing of the bone and not explainable by anterior deposition of periosteal new bone, as sometimes occurs in cases of treponemal disease. Jaffe (1972:921, 937) made the distinction between “true” and “pseudo” tibial bowing, thus making it possible to differentiate between conditions that cause it. Tibial bowing in rickets can resemble as a condition known as saber shin, or boomerang leg, which occurs in cases of Yaws; both diseases result in a true bowing of the tibia (Aufderheide and Rodríguez-Martín 1998:156; Ortner 2003:275). Syphilis results in the appearance of bowing, or pseudo-bowing, from periosteal new bone formation on the anterior of the tibia (Aufderheide and Rodríguez-Martín 1998:156).

In the following section, the prevalence of these lesions at Indian Knoll, as described by previous researchers, will be discussed. Of particular importance to the present study are periosteal lesions, which have been described in great detail in the Indian Knoll skeletal population.

## PRIOR BIOARCHAEOLOGICAL RESEARCH ON INDIAN KNOLL

The initial analysis of the remains was published by Webb in 1946 and included age and sex estimation of all skeletons for which preservation allowed. Further analysis was performed and published by Snow in 1948, which included evidence of infectious diseases and trauma. Since the original analysis by Snow (1948), Indian Knoll has been the subject of a number of bioarchaeological studies in relation to health and disease, but none have compared the presence of lesions among individuals to the extent that this project aims to do. One researcher (Nagy 2000) conducted a study of musculoskeletal stressors with regard to age, sex, and burial goods. Additionally, researchers (Cassidy 1972; Kelley 1980) have compared the prevalence of lesions at Indian Knoll with those of other populations. Additionally, some lesions have been compared between the sexes. Prior research on Indian Knoll, and some of the other Green River sites, is detailed below.

### *Abscesses*

Abscessing was compared to instances of arthritis in Indian Knoll by Snow (1948:502). Sixty-six males (25.0 percent) and 50 females (23.7 percent) exhibited abscessing and no evidence of arthritis (Snow 1948:502). Forty-four percent of males and 31.3 percent of females exhibited both arthritis and abscessing (Snow 1948:502).

Cassidy (1972:125) noted periodontal inflammations specifically, and differentiated between apical abscessing and localized inflammations, the latter of which was categorized as any destruction of the alveolar bone that was neither alveolar resorption associated with periodontal disease, nor apical abscessing (Cassidy 1972:117, 125). Apical abscesses were found in 25 of 87 (28.7 percent) adult males, and in 23 out of 69 (33.3 percent) adult females (Cassidy 1972:125). Localized inflammation was found in 6 males (6.9 percent) and 5 females (7.2 percent) (Cassidy 1972:125). Additionally, there were 22 males (25.3 percent) who exhibited both apical abscessing and local inflammation, and 11 females (15.9 percent) (Cassidy 1972:125).

The molars and premolars were the most common sites of both apical abscessing and inflammation in adult males, and in females with one exception: localized inflammations occurred second most frequently on canines followed by the premolars (Cassidy 1972:127). No significant difference in abscessing or periodontal inflammations was detected between males and females at Indian Knoll (Cassidy 1972:126). Kelley noted the presence of apical abscesses in 107 of 197 (54.3 percent) individuals at Indian Knoll, the highest total prevalence in his analysis of three sites (1980:128).

*Auditory Exostoses*

Snow (1948:509) noted the presence of bilateral exostoses in 45.0 percent of Indian Knoll males. Kelley also analyzed auditory exostoses, and stated that the condition disproportionately affected males: 3.3 percent of females exhibited the condition, while 38.5 percent of males had exostoses (Kelley 1980:131). Exostoses were most commonly located on the posterior wall, and Kelley stated that 10 of the cases were severe enough that they likely decreased hearing capacity (Kelley 1980:132). Additionally, Kelley

(1980:185) made the distinction between pedunculated and flat, broad exostoses in his population; he attributed the pedunculated lesions to a potentially genetic cause, and noted that they occurred in roughly 10.0 percent of his sample. Cassidy (1972:84) found bilateral exostoses that occluded most of the ear aperture in six males (6.7 percent).

Mensforth (2005:466) reported exostoses a number of Green River sites: 36.4 percent of 88 men at Carlston Annis, 51.6 percent of 254 men at Indian Knoll, 25.0 percent of 12 men at Kirkland, 21.3 percent of Barrett men, and 22.6 percent of Ward men. Significantly fewer females exhibited the condition, ranging from zero percent at Kirkland to 9.5 percent at Indian Knoll, with a total of 6.9 percent of Green River females exhibiting the condition (Mensforth 2005:466). An analysis by Herrmann (2002:127-133) found exostoses in 49 of 184 (26.6 percent) of crania at Carlston Annis, 15 of 70 (21.4 percent) at Read, 27 of 175 (15.4 percent) at Ward, 31 of 170 (18.2 percent) at Barrett, 15 of 55 (27.2 percent) at Chiggerville, and 165 of 495 (33.3 percent) at Indian Knoll. According to Mensforth (2005:466), the condition seems more common in the Green River region than other known populations.

The prevalence of auditory exostoses has been studied in great depth among shell midden populations. The Eva site in Tennessee had 3 cases of exostoses in 49 individuals (6.1%) and all were males (Lewis and Lewis 1961:154). An analysis by Wolf and Brooks (1979:917) indicated the presence of exostoses at the Rosenberger site, with the majority of cases occurring in males, and only one case in a subadult. Herrmann (2002:133) found them in 19 of 104 (18.2 percent) individuals sampled. Men appear to be more susceptible to exostoses based on a study by Mensforth (2005:466).

*Enamel Hypoplasia*

Cassidy (1972:139) noted that growth arrest lines suggesting that recurring, periodic episodes of nutritional stress at the site. The canines and third molars were scored by Cassidy when adults had at least 10 observable teeth, and the severity of linear enamel hypoplasia was considered in some of her analyses (1972:105-106). Enamel hypoplasia was observed in a large percentage of the Indian Knoll sample studied by Cassidy (1972:107), with 90.4 percent of adult males, and 86.0 percent of adult females exhibiting the trait in either the permanent third molar and/or canine. Based solely on data from the canines, 45 of 52 (86.5 percent) adult males had enamel hypoplasia, and 37 of 43 (86.0 percent) of females exhibited the defect. Males had lower rates of the condition when considering only the most severe cases: they had severe enamel hypoplasia in 2.1 percent of cases, and 2.3 percent of females exhibited severe hypoplasia (Cassidy 1972:110; Cassidy 1984:322). The majority of cases were mild: 59.6 percent in males and 53.5 percent in females exhibited shallow grooves or lines in the teeth, with 38.3 percent of males and 30.2 percent of females exhibiting deeper grooves (Cassidy 1972:110).

*Metabolic and Hematological Disorders*

Instances of metabolic conditions, such as scurvy or rickets, are rather uncommon in Archaic populations (Powell 1996:127). As for Indian Knoll, no cases of scurvy have been described by previous researchers, according to Powell (1996:127). Snow (1948:508) noted the presence of rickets in an eight month old at Indian Knoll as showing evidence of growth arrest lines and swelling distal ends of several elements. No cases of rickets were identified in Cassidy's sample population (1972:70), while Kelley (1980:99-

100) found only 7 cases in 345 individuals, though no evidence of tibial bowing was found in his analysis of Indian Knoll.

Snow (1948:498) first noted the presence of pinhole porosity on the calvarium, palate, and sphenoid. Cassidy (1972:71) explained that crania at the Hardin Village site exhibited thickened bone, discoloration, and small, irregular holes indicative of anemia, but Indian Knoll exhibited only minor lesions at lambda and in the orbits, with no evidence of thickening on the crania. However, Cassidy (1972:72) postulates that the lesions that may have been indicative of iron-deficient anemia, being cribra orbitalia and porotic hyperostosis, were too minor in her Indian Knoll sample to confidently say that they were pathological. Still, 31.7 percent of the sample exhibited porous lesions at Indian Knoll, with females affected slightly more often than males (Cassidy 1972:73). Kelley (1980:121) noted only 13 cases of anemia in his study of subadult skeletons, in the form of cribra orbitalia or porotic hyperostosis. Nine individuals analyzed by Kelley (1980:121) had cribra orbitalia, three had porotic hyperostosis, and only one individual exhibited the lesions of both conditions.

While scurvy was not diagnosed by Kelley (1980:102) in his analysis of Indian Knoll, he did note the presence of mild pitting on the parietals and occipitals of 41 individuals in his sample; of these, 28 were male and 13 female, and the majority were adults. It is known that the cranial lesions associated with scurvy may mimic anemia, and sometimes both conditions can occur simultaneously due to the presence of chronic bleeding in scorbutic individuals, and thus must be considered as a possible etiology in the present study. Ortner *et al.* (2001:343) note that scurvy is only rarely mentioned in analyses of archaeological remains, and because abnormal porosity can occur in multiple conditions, scurvy may be underrepresented in many archaeological sites.

*Periodontal Disease*

Cassidy stated that periodontitis was uncommon in her 1972 analysis of Indian Knoll (1984:324). Cassidy (1972) stated that the condition was not scored for specifically because the remains were from an archaeological context, and all inflammations were scored together. However, nine cases were specifically noted as occurring throughout the alveolus (Cassidy 1972:125). If we consider this as evidence of periodontal disease, the condition was unlikely based on the analysis by Cassidy.

Additionally, Cassidy (1972) did find that a significant percentage of the sample population had caries, exposed pulp chambers, and apical abscesses. Males and females were similarly affected by these conditions (Cassidy 1972:130). Dental caries occurred in adults at Indian Knoll, though the data compiled by Cassidy (1972:123) indicate they were infrequent occurrences per individual. Only 10.2 percent of individuals exhibited caries at Indian Knoll (Kelley 1980:126), evidence Kelley cited for possible increases in infection and disease in aging adults as nutrition decreased (1980:182).

Snow (1948:501, 530) commented on the state of attrition at Indian Knoll and attributed it to purely mechanical cause resulting from a heavy diet. When analyzed by Cassidy in 1972, over 56.0 percent of males, 63.2 percent of females, and 31.3 percent of adolescents exhibited either caries or pulp exposure (1972:123). Nearly 45.0 percent of males exhibited pulp exposures solely, along with 45.6 percent of females (Cassidy 1972:123). Similarly, Cassidy (1972:131) stated that extensive tooth wear took place over a short period of time in this population. Tooth loss gradually occurred with age on both the mandible and maxilla, without regard to sex (Cassidy 1972:129). Additionally, 15.5 percent (36 of 233) of individuals sampled by Kelley (1980:128) were edentulous on the mandible or maxilla, or both, exhibiting the highest frequency of any population in his sample. Similarly, in a study of three populations by Nealis (2011), Indian Knoll exhibited the highest rate of wear between the two Archaic populations analyzed (Nealis 2011:39).

In a study by Robbins (1977), severe periodontal disease with the presence of bone loss and abscessing was found to coincide with changes in the coronoid process. Robbins suggested that the individuals were adjusting their mastication habits, likely as the result of their dental conditions (Robbins 1977:16). A coarse diet is not the only cause of severe attrition, however. It has been stated that modern humans exhibit similar wear from using chewing tobacco (Robbins 1977:17). A study of attrition in multiple prehistoric populations sought to determine if the consumption of shellfish at Indian Knoll contributed to the severe attrition seen in individuals from the site (Nealis 2011). Nealis (2011) discovered that two populations, one of which may have consumed shellfish, were found to have utilized similar tools for food preparation, stone mortars and pestles, that likely incorporated grit into the diet, contributing to the presence of severe attrition in both populations (Nealis 2011:45-46). This result is evidence against the hypothesis held by researchers (Cassidy 1972:131; Powell 1996:126; Snow 1948:501, 530; Ward 2005:501) that shellfish contributed to the extreme attrition in the Indian Knoll population.

*Periosteal New Bone Formation*

Snow (1948:505-509) noted the presence of severe infection in several skeletons, and suggested that the diagnosis could be treponemal infection. Burial 490 at Indian Knoll was of particular interest to Snow (1948) due to the severity of inflammatory lesions. Snow described this individual as exhibiting remodeling on the frontal and around the nasal aperture, in addition to having extensive “lace-like” bone formation on a number of elements (Snow 1948:505).

Shermis (1974) analyzed periosteal reactions in a sample of 88 individuals from Indian Knoll, and suggested that they were possibly caused by the bacterium *Staphylococcus aureus*. Seven of the 88 individuals studied, or 7.9 percent, had periosteal reactions (Shermis 1974:145).

Cassidy noted bone infection rates at Indian Knoll, those of which were localized, but not traumatic, degenerative, nor congenital malformations (Cassidy 1972:85). Infection rates were compared between males and females by broad age categories (1972:86). Among males aged 17-29 years, the infection rate was 19.2 percent, and in the 30-39 year category the rate was 52.6 percent, and among those in the 40+ year category the rate was 47.8 percent (Cassidy 1972:86). Females exhibited significantly higher infection rates in the 17-29 year category at 39.3 percent (Cassidy 1972:86). Contrary to the rate in males, infection rates in middle adult females aged 30-39 years was lower at 20.0 percent (Cassidy 1972:86). Both males and females had similar rates of infection in the 40+ year category, ranging from 46.7 percent in females to 47.8 percent in males (Cassidy 1972:86).

Disseminated periosteal inflammations were scored separately from localized bone inflammations (Cassidy 1972). Cassidy’s (1972:89) analysis of 151 skeletons aged

17 years and older revealed that 3.6 percent had periosteal inflammation at the time of death, with another 4.8 percent in the age category with possible evidence of inflammation. Statistics were not calculated for individuals over the age of 30 years due to a small sample size (Cassidy 1972:90). Cassidy (1972:87) described the presence of striations that run along the long-axis of the long bones which coalesce into thicker deposits of new bone, and noted the presence of two conditions that occurred alone and simultaneously throughout the long bones: smooth billows and discolored plaques of new bone (Cassidy 1972:87). Cassidy (1972:87) goes on to note that these lesions are progressive, typically affecting one bone, and eventually spreading to other elements. Severity was scored by Cassidy (1972:88), with moderate cases being marked by the presence of multiple lesion sites outside of the tibia. Moderate lesions were found to be most common in those with lesions, though lesions of all severities occurred in 2.4 percent of the sample population (Cassidy 1972:88). Cassidy (1972:88) described possible lesions as mild striations on otherwise healthy bone, which accounted for 3.1 percent of periosteal lesions in the sample overall. The most likely cause of these disseminated periosteal inflammations, according to Cassidy (1972:92), was treponemal disease.

Kelley identified specific infection separately from non-specific infection. Kelley (1980:139) estimated that of all individuals he sampled, including subadults, 3.3 percent had non-specific periostitis, and 2.4 percent had non-specific osteomyelitis (Kelley 1980:140). Children were most affected by periostitis, followed by females (Kelley 1980:139). Osteomyelitis was observed in the exact opposite trend, affecting males disproportionately over females, with only one case among subadults (Kelley 1980:140).

The analysis concluded that 28 of 814 individuals (3.4 percent), had evidence of what may have been a specific infection, potentially treponemal disease (Kelley 1980:190-191). The majority of lesions in his sample population affected the nasals, palatines, and bones of the calvarium, while the long bones were a common site of lesions in children and infants (Kelley 1980:141).

Kelley noted a number of diseases can mimic the type of new bone formation associated with treponemal disease: Paget's disease, nonsuppurative osteomyelitis, tuberculosis, pyogenic osteomyelitis, myeloma, and metastatic carcinoma (Kelley 1980:145-146). Further, Kelley (1980:143) described the long bones in affected children as exhibiting a swollen diaphysis and tibial bowing, both of which can be indicative of congenital syphilis, but also of metabolic conditions such as rickets. The author goes on to note that the lesions themselves were diagnostic and different from those in the aforementioned list, and that the most likely etiology of the lesions was treponemal disease (Kelley 1980:146).

Cassidy (1972:91) postulated that there were several additional nutritional and infectious conditions that could lesions similar to those observed in Indian Knoll. Similarly, Kelley (1980) assessed the association between periosteal lesions and porotic hyperostosis in children at Indian Knoll, though no association was detected; the data revealed no cases where both lesions were present (1980:124). The majority of Kelley's (1980) sample was subadults, and of 50 individuals over the age of 10 years, one individual exhibited lesions of both cribra orbitalia and porotic hyperostosis (1980:121).

One consensus among those who have previously analyzed skeletal material from the Indian Knoll collection is the nature of some of the severe lesions observed at Indian

Knoll are indicative of treponemal infection (Snow 1948; Cassidy 1972; Kelley 1980). The presence of lesions among the Indian Knoll population which may be indicative of congenital syphilis is significant, as this may suggest that similar lesions exhibited by adults are evidence of healing or healed syphilitic lesions from childhood.

It is evident that Indian Knoll has been extensively analyzed with regard to nutrition and stress markers, and also with regard to infectious diseases. However, seldom has the association between conditions been made. Many of the conditions described above have one thing in common: inflammation. What happens when the body is faced with multiple sources of infection or stress and mounts multiple responses? Surely the same pathogens responsible for periodontal disease do not also induce bone formation in the tibia. By analyzing cases of multiple inflammations in individuals, we may be better able to understand the role of individual immune responses when evidence of nutritional stress markers and inflammatory lesions are present. A discussion of how evidence of inflammation has been linked to systemic inflammation immediately follows.

## LOCAL & SYSTEMIC INFLAMMATION

Evidence of inflammation in the Indian Knoll population has been described in detail by previous researchers. To learn more from these lesions, however, we must consider the etiology of inflammation, and what we can learn from it. Inflammation is a reaction produced by the immune system in response to tissue damage from a range of stimuli (Weston 2012:493). Inflammatory responses when infection is present are intended to contain and neutralize pathogens, and repair damage (Weston 2012:493). Inflammatory responses are generally local (Weston 2012:494), though some infections can produce a systemic effect. When the stimulus of inflammation lasts more than two weeks, the inflammatory response is characterized as chronic (Weston 2012:494).

When inflammation occurs in the body, it is typically confined to one location (Straub 2011:3; Weston 2012:494). However, inflammatory responses can be especially strong, and when this happens, a "spillover" of cytokines and activated cells can occur. Spillover of pro-inflammatory proteins in particularly severe inflammations is thought to be a leading force in systemic inflammatory diseases (Straub 2011:2-3). One of the primary mediators of inflammation is tumor necrosis factor (TNF), which can escape the local site of inflammation and enter the systemic circulation system (Streiter *et al.* 1993:S447-S448). The circulation of these proteins can have an impact on cell activation, and impact the physiology of the host (Streiter *et al.* 1993:S448). Abnormal immune responses have spurred researchers to note the potential of a “hyper-sensitive phenotype” (Shaddox *et al.* 2010).

Inflammatory responses on part of the immune system sometimes damage the body in the process of fighting pathogens. However, too weak of an immune response may not neutralize pathogens, while too strong of a response can result in increased inflammation and the display of pathologic symptoms in the host (Graham *et al.* 2005:382-383). Retention of an over-active immune response may be a reflection of cost versus benefit; similarly, it has been suggested that natural selection may favor a strong response despite the negative impacts it may induce on the host (Graham *et al.* 2005:382). It is has been hypothesized that altered cytokine levels occurring in cases of chronic infection may have a systemic impact on other infections in a host’s body (Stelekati and Wherry 2012). Barton and colleagues (2007) demonstrated that latent infection may confer immunity to other infections in a host, resulting from the cytokine environment (Barton *et al.* 2007:328). A similar study by Crespo and colleagues (2016) demonstrated that in individuals with chronic infections such as tuberculosis or leprosy, a robust immune response may be capable of altering the levels of inflammatory mediators, which could induce a hyper-inflammatory state in individuals who experience a subsequent infection, such as periodontal disease.

A number of factors may be conducive to a hyper-inflammatory phenotype, including genetics, environmental factors such as chronic infection, and systemic stress (Liston *et al.* 2016). Recent evidence confirms that up to 80 percent of variation in the human immune system can be explained by non-genetic factors (Liston *et al.* 2016: 637). For example, periodontal disease has been linked to cardiovascular conditions in a number of studies (e.g. Ajwani *et al.* 2003; Beck and Offenbacher 2005; DeStefano *et al.* 1993; Haq *et al.* 2015; Mattila *et al.* 1989). Haraszthy *et al.* (2000:1556) found that periodontal pathogens were present in atheromatous plaques. Periodontal disease has been hypothesized to associate with systemic inflammation, and is hypothesized to be linked to the proliferation of pro-inflammatory proteins (D'Aiuto *et al.* 2004:158-159; Loos *et al.* 2000:1533).

*Immune Cells and Bone Cells*

Turning now to osteological markers of inflammation, we begin to incorporate elements from the field of osteoimmunology. Osteoimmunology incorporates the study of the relationship between the immune system and skeleton, in addition to a number of related cellular interactions (Takayanagi 2007:292). Immune and bone cells share a number of regulatory molecules, which influence one another (Nakashima and Takayanagi 2009:555). All immune cells are derived from hematopoietic stem cells, and the bone marrow cavity provides the environment for the development of these cells (Walsh *et al.* 2006:34). The primary cells involved in the remodeling of bone are osteoclasts and osteoblasts, which absorb bone tissue and secrete bone matrix, respectively (Burns 2013:13). Similarly, osteoblasts form osteocytes when they become embedded in the matrix that they create (Burns 2013:13).

Osteoclastogenesis is regulated by an interaction of molecules including receptor activator of nuclear factor κβ ligand (RANKL), and RANK, which are the receptor molecules of RANKL (Klaus 2014:297). Further, T cells produce RANKL, conferring them the ability to induce osteoclastogenesis (Klaus 2014:297). Osteoclast differentiation is controlled by a number of cytokines which regulate the expression of RANKL and

RANK (Klaus 2014:297). In a study of mice, osteopetrosis and an interruption in tooth eruption was produced by a deficit of osteoclasts, due to the disruption of RANK and RANKL (Nakashima and Takayanagi 2009:556-557).

One cytokine that plays an important role in the proliferation of osteoclasts is TNFα (Takayanagi 2007:298). Additionally, cytokines such as IL-1 and TNFβ are known to promote the resorption of bone (Frost *et al.* 1997:91). Frost *et al.* (1997:94-95) demonstrated the proliferation of osteoblasts in the presence of three cytokines, IL-1β, TNFα, and TNFβ. Similarly, D'Aiuto and colleagues (2004:159) demonstrated that individuals who were homozygous for specific alleles, IL-1α and IL-6, were associated with higher CRP levels. IL-1β was found to promote the production of pre-osteoblasts, which form from mesenchymal stem cells (MSCs), when rats with tibial fractures were exposed to it, but reduced the production of MSCs (Lange *et al.* 2010:779-780). The ratio of cartilage to callus increased when rats were exposed to high levels of IL-1β, but the dose size of IL-1β had virtually no effect on callus volume, total cartilage volume, bone volume, bone to callus ratio, or cartilage to bone ratio (Lange *et al.* 2010:781). Similarly, it has been demonstrated that IL-1β promotes the healing of periodontal wounds but not dermal wounds (Graves *et al.* 2001). Studies such as these are important as they indicate the role of an individual's phenotype, and related cytokine expression, in response to inflammation.

*Archaeological Research on Systemic Inflammation*

Further study on inflammatory responses utilizing the archaeological record (DeWitte and Bekvalac 2011) demonstrated an association between periosteal lesions and periodontal disease, and has provided the inspiration for the present research design on the Indian Knoll population. The study conducted by DeWitte and Bekvalac (2011) analyzed human skeletal remains at the medieval St. Mary Graces cemetery in London, England. Statistical analysis was conducted to uncover associations between periosteal lesions and periodontal disease both with and without regard to age (DeWitte and Bekvalac 2011). Neither periosteal lesions nor periodontal disease were found to significantly associate with adult stature, meaning that childhood stress may not have played an important role in the presence of these lesions in adults at St. Mary Graces (DeWitte and Bekvalac 2011:613). However, periodontal disease did associate with the presence of enamel hypoplasia, and may indicate a risk factor with periodontal disease (DeWitte and Bekvalac 2011:613, 615). Periodontal disease and periosteal lesions were positively associated in the population, and age was positively associated with the presence of periodontal disease (DeWitte and Bekvalac 2011:613). DeWitte and Bekvalac (2011:615) postulate the possibility that periosteal lesions and periodontal disease may have been more likely to occur in individuals with nutritional deficiencies.

We cannot consider osteological markers to be indicators of the presence of a hyper-inflammatory phenotype (HIP). Rather, we must take any associations between bone lesions as evidence of a possible HIP, but caution must be used when attempting to explain the etiology of a HIP between osteological markers. Without soft tissues and blood to determine levels of pro-inflammatory proteins, we can only speak broadly to the possible mechanisms behind the inflammatory lesions in the present study's population.

# MATERIALS & METHODS

The research design of the present study was modeled after that of DeWitte and Bekvalac (2011); proxies that did not return significant results in the study by DeWitte and Bekvalac (2011) were eliminated for the present analysis. To test for similar associations across space and time in regards to systemic inflammation, a population from a different time period, geographic locale, and cultural association was chosen. Several additional markers of dietary stress were added to the research design to test for the possible association between overall health and inflammatory responses. Data collection was done with permission at the William S. Webb Museum of Anthropology at the University of Kentucky in Lexington, Kentucky.

*Metric Measurements and Photographs*

All measurements were taken with a set of sliding TESA stainless steel calipers with manual dial, to the nearest .01 mm. Photographs were taken with a Panasonic FH22 Lumix digital camera, with permission from the William S. Webb Museum of Anthropology.

*Selecting the Sample Population*

A computer generated pseudo-random sample population was drawn using a program developed in Java. The sample was stratified, and was not a true random sample. Based on the prevalence of periodontal inflammations and periosteal lesions described by previous researchers (Cassidy 1972; Kelley 1980; Shermis 1974), a sample size of 260 individuals was estimated to provide sufficient statistical power (Christina Pinkston, personal communication 2016) to detect an association between these lesions.

Age and sex estimations by Kelley (n.d.) were used in the sampling strategy. Age categories (Buikstra and Ubelaker 1994:9) were assigned for sampling purposes. A stratified sample population was drawn from two standard categories: young adults, and middle adults. Older adults were uncommon at Indian Knoll, and so individuals in this age category were collapsed into the middle adult category for sampling. Burial numbers were input into the program from each of the following categories: young adults, encompassing individuals between the age of 20-35 years, and middle/old adults, encompassing individuals over the age of 35.5 years. The program was designed to sample 100 middle/old age adults, and 160 young adults with equal parts of males and females in each age category. Age is discussed in a future section with regard to how it was coded to reflect age category in the present analysis (young, middle, older).

*Age Estimation*

Snow (1948) estimated age for the entirety of the Indian Knoll population. A reanalysis by Kelley (n.d.) increased the proportion of adults over the age of 30 years. Herrmann and Konigsberg (2002:246) re-analyzed the age at death of the Indian Knoll population utilizing Todd’s (1921a, 1921b) 10-stage pubic symphysis system, and the eight-stage system for the auricular surface by Lovejoy and colleagues (1985). However, these estimates by Herrmann and Konigsberg (2002) were not readily available to the author, and so several age estimation methods outlined in Buikstra and Ubelaker (1994) were employed in the present study.

Following the selection of a sample population, estimation of age was performed by the author based on an analysis of subjective traits, as described in Buikstra and Ubelaker (1994). Ideal methods of age estimation included the condition of the sacro-iliac auricular surface (Buikstra and Ubelaker 1994:25, after Lovejoy *et al.* 1985, Meindl and Lovejoy 1989) and pubic symphysis (Buikstra and Ubelaker 1994:22-24, after Brooks and Suchey 1990, McKern and Stewart 1957, Suchey and Katz 1986, and Todd 1921a, 1921b). Observation of the condition of sternal rib ends (Isçan *et al.* 1985) based on the illustrations in Burns (2013:71) were also utilized to assign age estimations. An age range of five years was assigned to most burials, though on occasion a smaller range of three years was assigned to well-preserved burials.

Additional traits were used and compiled when none of the above were observable, and wide age ranges were assigned to factor in a margin of error. Cranial suture closure (Buikstra and Ubelaker 1994:32-38, after Meindl and Lovejoy 1985) was used when the pelvis was poorly preserved, in conjunction with tooth wear. Tooth wear analysis included attrition based on the system by Brothwell (1981:72) and tooth eruption system (Buikstra and Ubelaker 1994:51, after Ubelaker 1989), with use of the latter to eliminate adolescents from the sample population. Due to problems with comparability (Hillson 2005:216) between the populations represented in current tooth wear analysis systems, this method was employed only in cases where preferred elements for age estimation were unobservable. The diet consumed by this population wore the teeth very heavily early in life, and thus tooth wear may not a reliable indicator of age in the Indian Knoll population. A broad range of was assigned to those individuals scored by either tooth wear or cranial suture closure.

For statistical analyses, an average age was calculated from the assigned ranges. In cases where age was assigned after an analysis of tooth wear or cranial suture closure, these burials were eliminated from the statistical sample since the estimation may not be completely accurate, and since too broad of a range was assigned, thus making it difficult to make assumptions about these individuals.

*Sex Estimation*

Sex was re-estimated after sampling, and was performed utilizing a number of standard methods outlined in Buikstra and Ubelaker (1994:16-21). Analysis of the os coxae included an examination and scoring of subpubic concavity, ridge on the medial aspect, and ventral arc (Buikstra and Ubelaker 1994:17, after Buikstra and Mielke 1985, and Phenice 1969). Additionally, presence of a preauricular sulcus (after Milner 1992), and the width of the greater sciatic notch were considered based on the sketches provided in Buikstra and Ubelaker (1994:18-19). Cranial features were also examined: sharpness supra-orbital margin, size of mental eminence, prominence of the supra-orbital ridge, size of the mastoid process, and the presence and size of a nuchal hook (Buikstra and Ubelaker 1994:20). All features were scored using the methods and descriptions provided in Buikstra and Ubelaker (1994), after Ascadi and Nemeskéri (1970).

All scores were considered, and an estimation of sex assigned to the best fitting category (see Buikstra and Ubelaker 1994:21). For statistical analyses involving sex, all individuals that fell in either “possible female” or “possible male” categories were eliminated. Eliminating individuals who were ambiguous with regard to sex was done to more accurately assess the effect of sex on the presence and absence of lesions.

*Statistical Analysis*

Two statistical tests were utilized in this research project: the Pearson Chi-Square test, and the Hierarchical Log Linear Analysis. The Pearson Chi-Square test is ideal for testing associations between categorical data in large sample sizes. Given the number of categories recorded for this analysis, and the representation of lesions associated with both nutritional deficiencies and infection, the chi-square test seemed the ideal test to compare between and within conditions. Given that the expected cell counts were expected to exceed a count of five, this test was deemed suitable for testing the conditions scored in the Indian Knoll population. The goal of utilizing this test was to analyze the correlation between nutritional deficiencies, between infectious lesions, and between infectious lesions and nutritional deficiencies.

In order to test the effects of age and sex on the presence of periodontal disease, the hierarchical log linear test was utilized. This test, like the Pearson chi-square test, tests the relationship between categorical and binary data. Like the Pearson chi-square test, counts of less five should comprise no more than 20 percent of cells, otherwise statistical power is lost. Backwards elimination was used to achieve the best model fit.

Due to the prevalence of minor lesions throughout the Indian Knoll population, a decision was made post-data collection to establish a minimum severity criterion for scoring individuals as "present" for the conditions previously described. In the following section, the scoring systems for each lesion are described, and the minimum severity criterions are discussed. It should be stated that the scoring systems applied here are subjective in nature, with "mild" lesions potentially being less mild than in other populations.

*Scoring Lesions*

A number of lesions were scored for the present study, with differentiations made for active, healed, and healing lesions. For the statistical analyses, however, all lesions were pooled regardless of their state of activity at the time of death. For the purpose of the present study, activity of the lesions was not as important as the presence of a lesion. Presence of a lesion was considered to be indicative of an inflammatory response. Additionally, it should be noted that the numeric scored detailed below did not factor into the coding of variables; that is, simple “presence” or “absence” was considered rather than the severity of lesions. Minimum severity criteria were established and are described when applicable.

*Scoring Abscesses.* Abscesses were commonly observed in the present study, even in individuals with incomplete dentition due to postmortem damage. Antemortem tooth loss was common, in addition to postmortem tooth loss and damage, and thus limited the number of observable samples. Available quadrants were scored for presence or absence. Resorption, either newly healing or mostly healed to the point of edentulism, covering the majority of the quadrant was scored as “unobservable” for abscessing since true presence or absence cannot be determined. Additionally, in cases where only one or two teeth had been lost and bone was mostly or completely healed over, a score of “unobservable” was given since it could not definitely be ruled out that the individual had suffered an abscess. Absence was noted only when complete quadrants did not display any signs of inflammation or damage. Incomplete, missing, and damaged quadrants were scored as “unobservable” since true absence cannot be determined from incomplete remains.

For statistical analysis, the right mandible was chosen as the portion to assess for lesions because it offered the largest sample of individuals with viable scores. Abscessing that was either lingual, buccal, or labial was considered if it was lateral to the neck of the tooth or adjacent to the root (apical). Abscessing of the maxillary sinuses and central palate were scored, but for statistical analyses these were not included if they represented the only instances of infection. Abscessing was scored as "present" when alveolar bone exhibited evidence of active, healing, or healed abscesses, and "absent" only when no extensive antemortem or postmortem damage had been sustained to the alveolar bone.

*Scoring Auditory Exostoses.* All bony nodules in the auditory canal were scored on both the left and right temporals, and scores were assigned based on the percentage of occlusion the exostosis caused to the auditory canal. It is possible that this broad distinction included osteomata rather than strictly exostoses. To eliminate the possibility of overestimating exostoses, only bilateral lesions were scored as "present" in the chi-square analysis. Size was not taken into account, and lesions were not eliminated from the analyses due to small size. A score of "absent" was given if only one canal was affected, or if both canals were free from lesions. The scoring system for auditory exostoses was adapted from Cook (1976:319) and was as follows:

Unobservable
Absent
1 – Up to 1/3 occluded
2 – Approximately 2/3 occluded
3 – 2/3+ occluded

*Scoring Enamel Hypoplasia.* Enamel hypoplasia was scored using the system described in Buikstra and Ubelaker (1994:56) and focused solely on the type of lesion:

Unobservable
Absent
"1 - Linear horizontal grooves
2 - Linear vertical grooves
3 - Linear horizontal pits

4 - Non-linear array of pits
5 - Single pits"

Due to the fact that opacities are rarely reported in archaeological contexts and often require the assistance of an ultraviolet light (Hillson 1996:171), these were not scored in the Indian Knoll population. Since only the matter of presence was important for the statistical analysis, measurement of the lesion to determine age of onset was not performed.

Individuals were scored for the presence of enamel hypoplasia on the anterior teeth: central incisors, lateral incisors, and canines. Individual remains of the mouth were often fragmented and complete dentition for any given individual was rare, and so a single tooth was used as the proxy for determining presence or absence of enamel hypoplasia. Only the scores from the left mandible canine were included in the statistical analyses. If the left mandibular canine was missing, too worn, or so severely damaged that it could not be scored, a score of "unobservable" was given. In many of the middle-adults and older-adults severe attrition was not uncommon: the teeth were worn beyond the crown and it was impossible to score for enamel hypoplasia. These cases were given a score of "unobservable."

This method did eliminate individuals from the testable population who may have exhibited evidence of enamel hypoplasia, but not on the left mandibular canine. Since individuals with complete dentition were uncommon, this was deemed the most suitable method to maximize the sample size, and determine true absence or presence in individuals with limited dentition. Additionally, the mandibular canines have a long period of development in the human body prior to eruption by age ten (Buikstra and

Ubelaker 1994:51; Hillson 2005:172), and provide information about physiological stress over a long period of time as compared to other teeth.

*Scoring Orbital Porosity and Cranial Porosity.* For the sake of clarification, the present study refers to lesions on the cranium as porotic hyperostosis and "cranial porosity," and to lesions of the orbits as cribra orbitalia and "orbital lesions."

Orbital porosity was scored based on the location of porosity, and severity of porosity. Both the left and right orbits were scored, though all cases of lesions, unilateral and bilateral, were included in the statistical analysis. The scoring system was adapted from Cook (1976:320) and was as follows:

Unobservable
Absent
1 – Superio-lateral wall porosity
2 – Superio-medial wall porosity
3 – Elevated with porosity
4 – As above with thickening

Porosity on the calvarium was discovered to be extremely common in this population, and so multiple scoring systems were combined to best score the location and severity of lesions in this population. The presence of porosity on virtually all skeletons in the sample population was a deciding factor against classifying all lesions as porotic hyperostosis. The scoring systems utilized scored the location of lesions, severity of porosity and new bone formation, and included a subjective category to estimate the degree of involvement on the entire external vault. Lesions of the cranium were mild in this population, and the subjective scores utilized in the present study may have scored normal variations as "lesions." The scoring system for type and severity of lesions on the calvarium was as follows, based loosely on the system by Buikstra and Ubelaker (1994:115):

Unobservable
Absent
1 – Porosity only
2 – Porosity with slight thickening
3 – Moderate as above
4 – Hair-on end/severe

The scoring system for location of lesions was scored based on the number of elements affected by lesions, with ascending numbers indicating placement on the calvarium, from front to back, rather than increased severity. Elements displaying the most severe lesions present in the individual were selected, and less severe lesions noted. The scoring system for location of lesions on the calvarium was as follows:

Unknown
Frontal
Left Parietal
Right Parietal
Occipital
Frontal and Parietal(s)
Frontal and Occipital
Left and Right Parietal
Left and Right Parietals and Occipital
All

The scoring system for total cranial involvement was a subjective scored assigned by the author, and was intended to score the portion of the total cranial affected by lesions, regardless of severity. All crania were scored regardless of preservation and fragments pieced together to obtain the best suitable score. However, a score of "unobservable" was given when the cranium was too fragmented to determine the total extent of lesions so as not to include them in the statistical analysis. "Mild" indicated that lesions covered roughly half of the external vault, with "Moderate" indicating roughly three-quarters coverage with lesions. “Extensive” indicates that individuals displayed lesions covering most of the external vault, if not all of it. This allowed the author to determine the total display of lesions across the calvarium to determine population trends, and to extract moderate and severe cases of extent to be used in the statistical analyses in conjunction with the above scoring system of severity. The subjective scoring system for total cranial involvement was as follows:

Unobservable
1 - Mild
2 - Moderate
3 - Extensive

Since most individuals displayed some sort of porosity on at least one bone, this allowed the author to exclude cases of normal variation, and to mark only those moderate and severe cases as “present” for cranial porosity in the statistical analysis. By combining scores from both severity and extent categories, it was also possible to eliminate those crania that were too incomplete to include in the statistical analysis. For a score of “present” in the statistical analysis, the severity following severity criterion was used:

Porosity only of moderate extent or greater
Porosity with slight thickening of moderate extent or greater
All cases of porosity with moderate thickening, no minimum extent
All cases of porosity with severe thickening, no minimum extent
All cases of extensive coverage

*Scoring Periodontal Disease*. Porosity of the alveolar bone and bone resorption were scored when observable. In some cases, the alveolar crest had sustained damaged, and scores were not taken. Scores for alveolar bone resorption were taken from as many molars and pre-molars in all four quadrants of the mouth as possible. In cases where no bone was observable due to postmortem damage, tooth loss, abscessing, or edentulism, a score of “unobservable” was given. Resorption was not scored on teeth that showed evidence of abscessing in order to prevent the inclusion of false positives; this is due to the fact that the tooth may have become loose due to bone loss from the localized infection rather than from periodontal disease.

Individual teeth were measured for resorption and recorded on the maxilla and mandible recording sheets from Buikstra and Ubelaker (1994). The threshold of 2 mm was chosen as the proxy for periodontal disease. Thus, scores above this threshold were taken to indicate evidence of periodontal disease even if not present on all teeth in one region. Those teeth with scores below 2 mm were taken to indicate the absence of periodontal disease.

For the statistical analysis, only scores from the molars of the left mandible were used because it provided a large number of viable scores. If at least one of three molars scored above 2 mm, a score of “presence” was indicated for that individual. This method was employed to maximize the sample size. Teeth were scored as “absent” of the condition if all three molars exhibited no evidence of alveolar resorption or damage, and were not missing.

The preliminary research design included the presence of porosity on the alveolar bone as an indicator of periodontal disease. However, due to its prevalence in the population, porosity was not utilized for the statistical analysis. Porosity was scored as present or absent above each tooth. Porosity around the site of an abscess was not considered a marker of periodontal disease, in these cases a score of “unobservable” was noted for the individual tooth. Porosity was only scored if the alveolar bone was free from abscessing, damage, and was not edentulous or in the process of remodeling.

*Scoring Periosteal Lesions*. The anterior diaphysis of the tibia was scored for evidence of periosteal lesions. In cases where lesions were present on the proximal, distal, or dorsal aspects, these were not scored and were not included in the statistical analyses. Both medial and lateral aspects to the anterior crest of the tibiae were considered "anterior" and included in the statistical analysis. Lesions, when present, were scored for size and severity in the form of thickness of the new bone formation and relative coverage of the anterior surface of the tibia. In cases where lesions extended beyond the anterior boundary and extended either dorsally or distally, only the anterior portion was scored. Tibiae that were incomplete or damaged were not scored as "absent" if lesions were not present, and rather assigned a score of "unobservable." The scoring system was created by the author, and adapted from multiple systems designed by Cook (1976:318). The scoring system was as follows:

Unobservable
Not present
1 - Thin striae to half
2 - Thin striae ½+
3 - Raised striae to half
4 - Raised striae ½+
5 - Isolated mild thickening
6 - Mild thickening to half
7 - Mild thickening ½+
8 - Isolated moderate thickening
9 - Moderate thickening to half
10 - Moderate thickening ½+
11 - Extremely thick to half
12 - Extremely thick ½+

For the purpose of statistical analysis, a decision was made to eliminate lesions that may be of a traumatic nature. Since subperiosteal hematomas resulting from trauma can resemble infectious lesions, it was deemed necessary to establish a minimum criterion for scoring an individual as "present" for periosteal lesions. Individuals exhibiting "isolated" lesions smaller than 3 cm in either length or width were not scored as "present" in an attempt to exclude small, traumatic lesions. Cases of thin striae on the tibiae (Figure 10), in which the striae were not raised because no new bone formation had occurred, were not considered to be "present" for lesions. That is, if an individual exhibited thin striae or isolated lesions, they were marked as being "absent" of periosteal lesions for the statistical analyses. Additionally, only cases where both tibiae exhibited lesions that met the minimum severity criterion were considered as "present" for periosteal lesions.


Figure 10. Thin striae extend along the medial aspect of a right tibia and continue on the posterior of the element. Photo by the author, used with permission.

*Scoring Bowing of the Tibiae.* Bowing of the tibiae was assessed based on the degree of involvement. The scoring system was modified from Cook (1976:319) and was as follows:

Unobservable
None
1 - Slight
2 – Marked

For use in the chi-square analysis, both categories indicating presence were collapsed into a single "presence" category. Only instances of bilateral bowing were considered as "presence" of the condition, and "absent" if bowing was only detected on one side. Tibiae were scored as "unobservable" if too little of the tibiae remained or was otherwise unable to be assessed.

*Coding Variables*

For use in SPSS, all scores were coded into a number. Sex was coded as male (1) and female (2), with all possible females and possible males eliminated from the sample to avoid including individuals of ambiguous sex. Lesions were scores as unobservable (0), absent (1), or present (2). Age was scored in two different ways for use in the chi-square test and hierarchical log linear analysis. For the chi-square analysis individuals were assigned to one of three age categories, equated in this analysis to young, middle, and older adult age: 20-30 years (1), 30.5-40 years (2), and 40.5+ years (3). For the hierarchical log linear analysis, and the chi-square test between periodontal disease and age, age was collapsed into two categories to increase cell counts: 20 to 35 years (1), and 35.5+ years (2).

RESULTS

The results of the statistical analyses performed in the present study are summarized in Table 2. For clarity, the results of the chi-square and hierarchical log linear analysis (HLLA) will be addressed separately, with the results summarized and then outlined in greater detail for the purpose of discussion. Only the results that were significant will be outlined in greater detail, and discussed in the next section.

| | **AB** | **Bow** | **CO** | **EH** | **Exo** | **PD** | **PH** | **PL** | **Age** | **Sex** |
|---|---|---|---|---|---|---|---|---|---|---|
| **AB** | X | X | X | X | X | B | X | NS | S | NS |
| **Bow** | | X | NS | X | X | X | NS | B | X | S |
| **CO** | | | X | NS | S | NS | B | NS | NS | S |
| **EH** | | | | X | NS | NS | NS | NS | NS | S |
| **Exo** | | | | | X | S | S | NS | S | S |
| **PD** | | | | | | X | NS | B | S | NS |
| **PH** | | | | | | | X | NS | NS | S |
| **PL** | | | | | | | | X | S | NS |
| **Age** | | | | | | | | | X | X |
| **Sex** | | | | | | | | | | X |

Table 2. Summary of results indicating significant and non-significant associations in the chi-square analyses.

**KEY TO RESULTS:**
B = Borderline significant
NS = Not significant
S = Significant
X = Test not performed

*Hierarchical Log Linear Analysis*

The hierarchical log linear analysis (HLLA) was utilized to determine whether a three-way association exists between periodontal disease (PD) of the left mandibular molars and bilateral lesions of the tibiae (PL), and whether that association is independent of age. Backwards elimination was used to determine the best model fit, and to eliminate interactions between variables that were insignificant. A significance criterion of .05 was used in this analysis. Since samples with observable dentition were few, and individuals over the age of 50 years with dentition were few, it was necessary to collapse age into two categories for this analysis, as mentioned previously.

The results of the HLLA indicated that the three-way interaction between variables was not significant (Table 3). When the effect of periosteal lesions was deleted, the two-way interaction between PD and age was significant ($p = < .05$). When the effect of PD was removed, PL and age were not significantly associated ($p = .693$). The association between PD and PL was significant ($p = .056$).

| **Interaction** | **Chi-square** | ***P* value** |
|---|---|---|
| PD X PL X Age | .000 (df 1) | .997 |
| PD X Age | 13.699 (df 1) | < .05 (.000) |
| PL X Age | .156 (df 1) | .693 |
| PD X PL | 3.664 (df 1) | .056 |

Table 3. Hierarchical log linear analysis results for PD, PL, and age.

The lack of a three-way association was the expected result, but it is possible that low cell counts, due to limited samples, may have reduced the power of this model. Despite this, the association between periodontal disease and periosteal lesions existed in the absence of age, which may indicate a heightened immune response in those individuals exhibiting both lesions. It is important to note, however, that the chi-square results, which will be discussed in the following section, indicated an association with regard to age and periosteal lesions when age was expanded into three categories.

*Chi-square Analysis*

The chi-square analysis proved to be a suitable test for analyzing the associations between two lesions in the Indian Knoll sample population. A number of associations were detected between lesions, though not all associations were positive. In some cases, the presence of one lesion was more likely to result in the absence of another lesion. Additionally, age and sex were tested against all lesions. As mentioned previously, three age categories were used to test for an age effect in all chi-square tests, with the exception being age by periodontal disease.

*Significant Age and Sex Associations.* The results of the chi-square tests with regard to age and sex are summarized in Table 4. Additionally, the results of each association are detailed in Tables 5-13, and are described in greater detail.

| **Significant age and sex associations** | |
|---|---|
| **Significant age associations** | **Chi-square results** |
| Age X abscessing | $\chi^2 = 37.614$; df 2; $P = < .05$ (.000) |
| Age X exostoses | $\chi^2 = 6.087$; df 2; $P = .048$ |
| Age X periodontal disease | $\chi^2 = 7.568$; df 1; $P = .006$ |
| Age X periosteal lesions | $\chi^2 = 12.768$; df 2; $P = .002$ |
| | |
| **Significant sex associations** | **Chi-square results** |
| Sex X cribra orbitalia | $\chi^2 = 13.062$; df 1; $P = < .05$ (.000) |
| Sex X enamel hypoplasia | $\chi^2 = 5.901$; df 1; $P = .015$ |
| Sex X exostoses | $\chi^2 = 32.588$; df 1; $P = < .05$ (.000) |
| Sex X porotic hyperostosis | $\chi^2 = 42.619$; df 1; $P = < .05$ (.000) |
| Sex X tibial bowing | $\chi^2 = 8.094$; df 1; $P = .004$ |

Table 4. Table summarizing the age and sex associations detected in the present study.

Two chi-square tests detected age effects that were expected: age and periodontal disease, and age abscessing. The results of the chi-square test involving age demonstrated that abscessing of the right mandibular increased sequentially with age (Table 5). The majority of individuals in both middle and older adult age exhibited evidence of abscessing. Similarly, the majority of individuals in middle/older adult age exhibited alveolar resorption (Table 7), which may be indicative of periodontal disease. In this test, a larger proportion of middle/older adults than young adults exhibited evidence of resorption.

The results of the chi-square test involving age and exostoses detected a similar trend to what other researchers have reported. Exostoses occurred most frequently in individuals of middle adult age, and slightly less so in older adult age (Table 6). This result is similar to the results noted in individuals from the Rosenberger site in Tennessee (Wolf and Brooks 1979:917).

Contrary to the results from the HLLA described previously, periosteal lesions and age were positively associated in the chi-square test. More middle adults exhibited bilateral periosteal lesions of the tibiae in this analysis (Table 8). However, the presence of periosteal lesions was not age progressive in the sample population. Similar percentages of middle and older adults exhibited lesions. Thus, it is possible that the presence of periosteal lesions is positively associated with age in the Indian Knoll population.

| Age X abscessing | | | |
|---|---|---|---|
| | **No abscessing** | **Abscessing** | **Total** |
| **20-30 years** | 59 (76.6%) | 18 (23.4%) | 77 |
| **30.5-40 years** | 28 (33.7%) | 55 (66.3%) | 83 |
| **40.5+ years** | 7 (25.0%) | 21 (75.0%) | 28 |
| **Total** | 94 | 94 | 188 |

Table 5. Results of the chi-square test between age and abscessing of the right mandible.

| Age X exostoses | | | |
|---|---|---|---|
| | **No exostoses** | **Exostoses** | **Total** |
| **20-30 years** | 70 (88.6%) | 9 (11.4%) | 79 |
| **30.5-40 years** | 71 (74.0%) | 25 (26.0%) | 96 |
| **40.5+ years** | 33 (76.7%) | 10 (23.3%) | 43 |
| **Total** | 174 | 44 | 218 |

Table 6. Results of the chi-square test between age and bilateral auditory exostoses.

| Age X periodontal disease (PD) | | | |
|---|---|---|---|
| | **No PD** | **PD** | **Total** |
| **20-35 years** | 29 (51.8%) | 27 (48.2%) | 56 |
| **35.5+ years** | 1 (8.3%) | 11 (91.7%) | 12 |
| **Total** | 30 | 38 | 68 |

Table 7. Results of the chi-square test between age and alveolar resorption below the left mandibular molars.

| Age X periosteal lesions (PL) | | | |
|---|---|---|---|
| | **No PL** | **PL** | **Total** |
| **20-30 years** | 59 (78.7%) | 16 (21.3%) | 75 |
| **30.5-40 years** | 46 (52.3%) | 42 (47.7%) | 88 |
| **40.5+ years** | 23 (57.5%) | 17 (42.5%) | 40 |
| **Total** | 128 | 75 | 203 |

Table 8. Results of the chi-square test between age and bilateral lesions of the tibiae.

A number of associations were detected in which sex had an effect on the presence of lesions. For example, females exhibited cribra orbitalia almost twice as often as males (Table 9). However, the opposite effect was detected with porotic hyperostosis: more males exhibited porosity and slight new bone formation than females (Table 12). Roughly three times as many men exhibited evidence of porotic hyperostosis as females.

Additionally, males exhibited enamel hypoplasia of the left mandibular canine more often than females did (Table 10). Males also exhibited bilateral exostoses more than females (Table 11): roughly one third of males exhibited exostoses, compared with only four percent of females. The results of the chi-square test between tibial bowing and sex indicated that females were disproportionately affected by the condition. More females exhibited bilateral bowing of the tibiae than males (Table 13). The results of these chi-square tests between lesions and sex may indicate that nutritional deficiencies, or potentially infections, disproportionately affected the sexes.

| **Sex X cribra orbitalia (CO)** | | | |
|---|---|---|---|
| | **No CO** | **CO** | **Total** |
| **Male** | 68 (73.1%) | 25 (26.9%) | 93 |
| **Female** | 46 (47.4%) | 51 (52.6%) | 97 |
| **Total** | 114 | 76 | 190 |

Table 9. Results of the chi-square test between sex and cribra orbitalia.

| **Sex X enamel hypoplasia (EH)** | | | |
|---|---|---|---|
| | **No EH** | **EH** | **Total** |
| **Male** | 19 (32.8%) | 39 (67.2%) | 58 |
| **Female** | 28 (56.0%) | 22 (44.0%) | 50 |
| **Total** | 47 | 61 | 108 |

Table 10. Results of the chi-square test between sex and enamel hypoplasia of the left mandibular canine.

| **Sex X exostoses** | | | |
|---|---|---|---|
| | **No exostoses** | **Exostoses** | **Total** |
| **Male** | 64 (63.4%) | 37 (36.6%) | 101 |
| **Female** | 95 (96.0%) | 4 (4.0%) | 99 |
| **Total** | 159 | 41 | 200 |

Table 11. Results of the chi-square test between sex and bilateral auditory exostoses.

| Sex X porotic hyperostosis (PH) | | | |
|---|---|---|---|
| | **No PH** | **PH** | **Total** |
| **Male** | 29 (33.7%) | 57 (66.3%) | 86 |
| **Female** | 74 (82.2%) | 16 (17.8%) | 90 |
| **Total** | 103 | 73 | 176 |

Table 12. Results of the chi-square test between sex and porotic hyperostosis.

| Sex X tibial bowing | | | |
|---|---|---|---|
| | **No bowing** | **Bowing** | **Total** |
| **Male** | 76 (85.4%) | 13 (14.6%) | 89 |
| **Female** | 62 (67.4%) | 30 (32.6%) | 92 |
| **Total** | 138 | 43 | 181 |

Table 13. Results of the chi-square test between sex and bilateral bowing of the tibiae.

*Significant and Borderline Associations between Lesions.* All other significant associations detected between lesions using chi-square tests are summarized in Table 14. A number of significant associations between lesions were detected, but as mentioned previously, not all associations were positive. In some cases, the majority of individuals with a lesion were absent of another lesion. Specific details of these tests are outlined in Tables 15-21.

| Significant and borderline associations between lesions | |
|---|---|
| **Significant associations** | **Chi-Square results** |
| Exostoses X cribra orbitalia | $\chi^2 = 8.510$; df 1; $P = .004$ |
| Exostoses X periodontal disease | $\chi^2 = 4.632$, df 1; $P = .031$ |
| Exostoses X porotic hyperostosis | $\chi^2 = 4.411$, df 1; $P = .036$ |
| | |
| **Borderline associations** | **Chi-square results** |
| Abscessing X periodontal disease | $\chi^2 = 3.032$; df 1; $P = .082$ |
| Cribra orbitalia X porotic hyperostosis | $\chi^2 = 3.534$; df 1; $P = .060$ |
| Periosteal lesions X periodontal disease | $\chi^2 = 3.524$; df 1; $P = .061$ |
| Tibial bowing X periosteal lesions | $\chi^2 = 3.055$; df 1; $P = .080$ |

Table 14. Summary of the significant and borderline significant associations detected between lesions in the present study.

Individuals exhibiting bilateral auditory exostoses were more likely not to have cribra orbitalia (Table 15). Only 22.0 percent of the individuals with exostoses exhibited cribra orbitalia. Of the individuals who exhibited exostoses, over 83.0 percent also exhibited alveolar resorption (Table 16). Additionally, over 50.0 percent of those with exostoses exhibited porotic hyperostosis (Table 17), meaning that individuals with exostoses were more likely to have porotic hyperostosis than not, but not by a large margin.

| **Exostoses X cribra orbitalia (CO)** | | | |
|---|---|---|---|
| | **No CO** | **CO** | **Total** |
| **No exostoses** | 84 (52.8%) | 75 (47.2%) | 159 |
| **Exostoses** | 32 (78.0%) | 9 (22.0%) | 41 |
| **Total** | 116 | 84 | 200 |

Table 15. Results of the chi-square test between bilateral auditory exostoses and cribra orbitalia.

| **Exostoses X PD** | | | |
|---|---|---|---|
| | **No PD** | **PD** | **Total** |
| **No exostoses** | 26 (51.0%) | 25 (49.0%) | 51 |
| **Exostoses** | 2 (16.7%) | 10 (83.3%) | 12 |
| **Total** | 28 | 35 | 63 |

Table 16. Results of the chi-square test between bilateral auditory exostoses and alveolar resorption below the left mandibular molars.

| **Exostoses X porotic hyperostosis (PH)** | | | |
|---|---|---|---|
| | **No PH** | **PH** | **Total** |
| **No exostoses** | 95 (64.6%) | 52 (35.4%) | 147 |
| **Exostoses** | 18 (46.2%) | 21 (53.8%) | 39 |
| **Total** | 113 | 73 | 186 |

Table 17. Results of the chi-square test between bilateral auditory exostoses and porotic hyperostosis.

Several borderline associations were detected in which the $p$ value fell right on or just over the .05 significance criterion established for this analysis. Despite this, these associations were considered significant and will be discussed in greater detail. For example, the majority of individuals with abscessing of the right mandible also exhibited alveolar resorption below the left mandibular molars (Table 18). Though these etiologies are not related, this may be explained by the absence of oral hygiene practices at Indian Knoll.

Additionally, the majority of those with periosteal lesions of the tibiae also exhibited alveolar resorption below the left mandibular molars (Table 20). This may be the result of a systemic inflammatory response when a subsequent inflammation was experienced. If we consider the association detected between periosteal lesions and bowing of the tibiae, it may be possible that an inflammatory agent did not produce bowing of the tibiae; of those individuals that exhibited bowing the tibiae, less than half had bilateral periosteal lesions (Table 21).

Similarly, around one third of the individuals with cribra orbitalia also exhibited porotic hyperostosis (Table 19). The etiologies of these lesions may not be the same, which may explain why the majority of individuals with cribra orbitalia do not also exhibit porotic hyperostosis.

| **Abscessing X periodontal disease (PD)** | | | |
|---|---|---|---|
| | **No PD** | **PD** | **Total** |
| **No abscessing** | 23 (53.5%) | 20 (46.5%) | 43 |
| **Abscessing** | 6 (30.0%) | 14 (70.0%) | 20 |
| **Total** | 29 | 34 | 63 |

Table 18. Results of the chi-square test between abscessing of the right mandible and alveolar resorption below the left mandibular molars.

| Cribra orbitalia (CO) X porotic hyperostosis (PH) | | | |
|---|---|---|---|
| | **No PH** | **PH** | **Total** |
| **No CO** | 61 (55.0%) | 50 (45.0%) | 111 |
| **CO** | 47 (69.1%) | 21 (30.9%) | 68 |
| **Total** | 108 | 71 | 179 |

Table 19. Results of the chi-square test between cribra orbitalia and porotic hyperostosis.

| Periosteal lesions (PL) X Periodontal disease (PD) | | | |
|---|---|---|---|
| | **No PD** | **PD** | **Total** |
| **No PL** | 22 (50.0%) | 22 (50.0%) | 44 |
| **PL** | 5 (25.0%) | 15 (75.0%) | 20 |
| **Total** | 24 | 37 | 64 |

Table 20. Results of the chi-square test between bilateral lesions of the tibiae and alveolar resorption below the left mandibular molars.

| Tibial bowing X periosteal lesions (PL) | | | |
|---|---|---|---|
| | **No PL** | **PL** | **Total** |
| **No bowing** | 97 (65.5%) | 51 (34.5%) | 148 |
| **Bowing** | 23 (51.1%) | 22 (48.9%) | 45 |
| **Total** | 120 | 73 | 193 |

Table 21. Results of the chi-square test between bilateral bowing of the tibiae and bilateral lesions of the tibiae.

All other interactions tested in chi-squares were not significant. Further discussion into the results of these analyses, and the implications of such, immediately follows.

# DISCUSSION

An in depth discussion of the associations detected in the present study, and inferences derived from these results, follows. For the sake of clarity, the hierarchical log linear analysis results between PD, PL, and age, and the related chi-square tests involving these lesions, are addressed first. Implications of the remaining chi-square tests follows.

*Periosteal Lesions, Periodontal Disease, and Age*

As proposed above, an immunological “shift” leading to systemic inflammation could have an impact on common chronic infections such as those generated by *Porphyromonas gingivalis* in periodontal disease, and *Staphylococcus aureus* in periosteal lesions. Based on the results of the present study, both periodontal disease (PD) and periosteal lesions (PL) may be good proxies to detect systemic inflammation.

Similar to the association detected by DeWitte and Bekvalac (2011) in the St. Mary Graces cemetery, an association between periodontal disease and periosteal lesions was detected at Indian Knoll. The results of the hierarchical log linear analysis (HLLA) did not produce a three-way association, meaning that the association detected between PD and PL may be independent of age at Indian Knoll. However, this assumption was contradicted by the chi-square test between PL and age (three categories), which indicated a positive association. Thus, the association between PD and PL at Indian Knoll may not have been independent of age. Regardless, the results of the associations detected in the HLLA and chi-square tests are worthy of further discussion.

As noted previously, the three-way association between PL, PD, and age was not significant, which may be indicative of a systemic inflammatory response in some individuals at Indian Knoll. However, the chi-square test between age (three categories) and bilateral lesions of the tibiae returned a significant $p$-value of .002. Periodontal disease and age (two categories) were also significantly associated in the chi-square analysis with a $p$-value of .006, and the association between PD and PL was just slightly over the significance criterion established for this study (.05) at $p = .061$. Older age has been associated with alveolar bone loss in periodontal disease, with shifting immune responses the cause (Liang *et al.* 2010:4). Thus, the trend observed in the present study of Indian Knoll is not unusual. However, given the positive association between age and PD, and between age and PL, in the chi-square analysis, it is possible that the hierarchical log linear analysis suffered from insufficient cell counts, and thus reduced power of the model. Therefore, it is possible that an age effect is present with PD and PL at Indian Knoll, which may not be indicative of a hyper-inflammatory trait.

Additionally, it is wise to consider the possibility that the criterion for designating the "presence" of periodontal disease may have led to an overestimation of the condition in the Indian Knoll sample, especially given the fact that Cassidy (1984) stated that periodontitis was uncommon at Indian Knoll. However, the association between PD and PL seems to indicate that some sort of inflammatory process was present in the mouth and tibiae, and that together these conditions may have induced a hyper-inflammatory state in some individuals.

While we cannot conclusively confirm or refute the presence of such a trait in the Indian Knoll population without living tissue and clinical analysis, the associations observed in the present study nonetheless contribute to the ongoing discussion between immune response and inflammatory lesions. It is possible that the lesions present in cases of periodontal disease and periosteal lesions are good proxies for detecting systemic inflammation in skeletal populations.

*Age and Sex Associations*

In addition to the association between age and PD, and age and PL, an age effect was also seen with abscessing. It has been hypothesized that the age effect in PD may be explained by the inflammatory status of an individual (Benatti *et al.* 2009). It is possible that a similar effect exists in the presence of abscessing within the Indian Knoll population. Further, this age effect may be explained by prolonged absence of oral hygiene practices.

Additionally, differences in the frequency of lesions between the sexes were detected at Indian Knoll, indicating that some males and females were disproportionately affected by lesions. As Nagy (2000:278-279) demonstrated that musculoskeletal markers were different between the sexes, so too were a few of the lesions analyzed in the present study. Exostoses were positively associated with both age and sex. As mentioned previously, males were disproportionately affected over females, which was not surprising. Further, exostoses were observed to occur most frequently in those of middle adulthood, and slightly less so in older adult age. This result was concurrent with findings at the Rosenberger site in Tennessee (Wolf and Brooks 1979:917). These results speak to the division of labor and gender roles at Indian Knoll, and may indicate the age at which the harvesting of shellfish was taking place. Further, the duration of time it takes to develop exostoses must be considered; it is possible that recurring irritation to the auditory canal over a number of years, starting in early adulthood, produced a higher prevalence of middle-aged adults with exostoses, due to the time it takes to form these bony growths. This association may also be influenced by the likelihood of individuals to survive into older adulthood, which may be why fewer older adults were affected by exostoses at Indian Knoll.

Further, ethnographic data has revealed that shellfish are generally harvested seasonally, with summer months popular gathering times for many groups (May 2005:79). Women are most involved in the gathering of shellfish, though men are more likely to venture to the shellfish beds that are located the farthest from camp (May 2005:79). Claassen (2005:291) estimates that shellfish harvesting at the DeWeese Mound site took place during warm months, probably summer and fall, based on a growth line analysis of shells, and suggests that this might indicate that warm weather harvesting was intended to provide food for winter storage. This coincides roughly with the ideal time for harvesting in the Ohio River - spring and summer - due to the warming water temperatures (Claassen 2005:287). Therefore, it is possible that despite the potential for warm-weather gathering, cool waters and wind encountered in the spring and fall months may have contributed to the formation of exostoses in men at Indian Knoll.

Additionally, orbital porosity disproportionately affected females over males at Indian Knoll, whereas enamel hypoplasia disproportionately affected males. In Cassidy's (1972) analysis of Indian Knoll, slightly more males exhibited enamel hypoplasia than females, so the result in the present study is similar. However, a much larger margin between males and females was detected presently, which may be due to differences in sampling.

In Cassidy's (1972) analysis, porous lesions on the cranium were seen most often in females. In the present study, females exhibited cribra orbitalia more often than males. However, an opposite trend was observed with porotic hyperostosis, which disproportionately affected males. If we assume that the lesions in the orbits are indicative of anemia, it is possible that females of child-bearing age at Indian Knoll would exhibit the lesion in higher numbers. Anemia can be caused by heavy bleeding in menstruating females (Ortner *et al.* 2001:343). Further, porotic hyperostosis was not significantly associated with age in this study. However, a chi-square test between porotic hyperostosis, age, and sex revealed that the lesion frequency increased with age among males (results not tabulated). Thus, the lesions associated with porotic hyperostosis and cribra orbitalia may be due to age related changes, with different effects seen in males and females at Indian Knoll. Without a more detailed microscopic analysis, we cannot say for certain what caused the cranial and orbital lesions in the Indian Knoll population. These results may be attributed to the sample population selected, and not a true effect in the entire population.

Lastly, men were less likely to be affected by bowing of the tibiae than females. If we consider that rickets can cause bowing of the tibia, it may possible that females were disproportionately affected by this nutritional deficiency than males at Indian Knoll. On the contrary, perhaps the trait is a relatively common, normal variation within females at Indian Knoll. However, given the significant association between periosteal lesions and bowing of the tibiae, normal variation does not seem a likely explanation.

*Borderline Significant Associations between Lesions*

Several chi-square tests in the present study resulted in a borderline significant *p*-value, meaning that the *p* value fell just outside of the significance criterion (.05) established for this analysis. These associations were: periosteal lesions (PL) by tibial bowing ($p = .080$), periodontal disease (PD) by abscessing ($p = .082$), orbital porosity by cranial porosity ($p = .060$), and periodontal disease by periosteal lesions, which was discussed above.

The borderline significant association detected between PL and tibial bowing may speak to the infectious nature of the periosteal new bone formation scored in this analysis, or the role of childhood nutritional deficiencies in later expressions of inflammation (i.e. reduced immunocompetence). If we consider the association between anterior tibial bowing and periosteal lesions in the Indian Knoll sample, it is possible that the bowing of the tibiae might be indicative of a nutritional deficiency such as rickets, and the PL indicative of scurvy. This would indicate a widespread trend of nutritional deficiencies in the Indian Knoll population. However, if we consider that infection may be the most likely cause of these lesions, we may cannot eliminate the possibility that the lesions are the result of treponemal disease, resulting in a mild expression of bowing. However, the majority of individuals with bowing did not have periosteal lesions, though this result was marginal. Additional full-body analyses with careful attention to diagnosis could illuminate the etiologies of these conditions.

Abscessing and PD were also associated at Indian Knoll. Individuals with abscessing were more likely to have alveolar resorption in a different part of the mouth (left mandible). It is possible that this is evidence of a hyper-inflammatory trait. It is possible that periodontal pathogens may play a role in the proliferation of multiple inflammatory conditions in the mouth, seen in the presence of both abscessing and alveolar resorption.

Lastly, the chi-square test between cribra orbitalia and porotic hyperostosis indicated that less than one third of individuals with orbital porosity also exhibited lesions on the cranium. It is possible that the etiologies are different between these lesions, hence why they do not occur simultaneously in most individuals.

*Metabolic or Hematological Conditions, or Infection on the Cranium?*

Cranial lesions in this population varied, but very few could be considered "moderate" in their expressions, considering the extent of bone formation that these lesions can achieve in other populations. Scores for this population were subjective, as there was a clear distinction between very mild cases and those involving more bone formation, which were marked as "moderate." Those lesions most marked in the Indian Knoll population may have been indicative of some form of infection. Several individuals exhibited lesions resembling a very mild form of *caries sicca*, in which shallow pits of eroding bone spotted the external vault. These individuals were eliminated from the statistical sample since the lesions do not resemble those associated with porotic hyperostosis, and because they obscured an assessment of lesions indicative of porotic hyperostosis. However, the presence of such lesions may be indicative of an infectious disease such as syphilis in the population, and it is possible that the lesions scored as porotic hyperostosis were early evidence of an infectious etiology, rather than hematological or metabolic in nature. However, the lack of an association between PH and lesions indicative of infection (e.g. periosteal lesions, periodontal disease) may indicate that these lesions were not of an infectious nature.

The majority of cranial lesions observed in this population occurred on at least the parietals, but frontal and occipital involvement was also relatively common, the latter more so. Porosity on the cranium was extremely common in this population and was found across all elements. The resemblance of some crania to an orange peel (see Sinnott 2013:102) leads one to think that scurvy may have been present in this population. If we consider the diet of Indian Knoll, it has been demonstrated that gastropods are high in calcium and iron, but contain virtually no ascorbic acid (Parmalee and Klippel 1974:808-809). Coupled with the lack of foods high in ascorbic acid during some seasons, scurvy cannot be ruled out. While this condition was not scored for specifically, its presence at Indian Knoll cannot be confirmed or refuted by the present study.

Due to the nature of porosity on the orbital roofs, these lesions may be indicative of an age factor in the presence and location of lesions. The majority of cases of orbital porosity in the Indian Knoll population were very minor, with no raised bone or hair-on end manifestations. These lesions might be the remnants of a childhood deficiency or age related change. The lesions of the external vault were much more marked by comparison, exhibiting extensive porosity and light new bone formation. Perhaps the lesions of the calvarium were developed in adulthood whereas orbital lesions are remnants of a childhood inflammation. As Cassidy (1972:72) noted, even cases of potential nutritional deficiency were not severe enough to conclusively say that they were pathologic. It is possible that these lesions are evidence of pseudo-pathology, age-related changes, or that the lesions of the vault were of an infectious nature, as mentioned above.

If we consider an infectious etiology of cranial lesions, the unusual porosity and light new bone formation observed does not seem to be a good indicator of systemic inflammation in the Indian Knoll population. Additionally, if these lesions are indicative of a deficiency, such as scurvy or anemia, they did not play a significant role in altering the expression of inflammation in other parts of the body. As mentioned before, a full-body analysis to differentiate the etiologies of these lesions, in addition to a microscopic analysis of them, would have been ideal had the intent of the present study been to diagnose. Although the present study did not seek to diagnose the condition, or conditions, that produced cribra orbitalia and porotic hyperostosis, it may be possible to eliminate a few causes based on the borderline significant association between them. If both conditions were evidence of the same nutritional deficiency, we might have seen more individuals with both orbital and cranial porosity. Similarly, if we consider that these lesions may have been age related changes, we might have seen equal proportions of males and females with both conditions. It is possible that these lesions in the Indian Knoll population are indicative of different etiologies, or that age related changes were different between males and females at Indian Knoll.

*Exostoses*

The chi-square tests involving auditory exostoses returned a number of significant associations: exostoses and cribra orbitalia, exostoses and porotic hyperostosis, and exostoses and periodontal disease. The majority of individuals with exostoses were likely to exhibit porotic hyperostosis. This may be due to the fact than more men at Indian Knoll exhibited both of these conditions. Additionally, individuals with exostoses were more likely to exhibit alveolar resorption. However, the opposite trend was true in the association between exostoses and cribra orbitalia, where only 22.0 percent of individuals with exostoses had orbital lesions. The association between periodontal disease and exostoses may be indicative of a systemic inflammatory response.

*Periosteal Lesions*

It is possible that the periosteal lesions recorded and analyzed in the present study can be attributed to both a traumatic and infectious cause. The tibia is not well covered by tissue (Larsen 2015:88) and is thereby exposed to trauma during physical activities. One can receive an injury to the tibia and break the skin, allowing bacteria to enter and cause periosteal inflammation (Roberts and Manchester 1995:129). This confuses the boundary between trauma and infectious lesions. In the present study, the author attempted to eliminate cases of mild trauma from the "presence" category by excluding periosteal lesions smaller than 3 cm in either direction. This was done to include as many cases of infection as possible, and eliminate cases of isolated, minor traumatic injuries. Similarly, it is possible that this criterion still included cases of traumatic subperiosteal hematomas, which could be indicative of scurvy.

It should be noted that many of the individuals at Indian Knoll did not exhibit severe lesions. It must be considered that because *Staphylococcus* accounts for roughly 90.0 percent of periosteal lesions (Ortner 2003:181), this pathogen may be behind the majority of tibial lesions in the Indian Knoll population. Paired with the fact that most individuals with bowing of the tibiae did not exhibit periosteal lesions in the present study, we may not be looking at widespread treponemal infection at Indian Knoll. One interesting study to consider here is that of Smith (2006:214), who concluded that having no cases of saber shin in the middle and late Archaic samples is evidence of a shift in morbidity into the early Woodland period, which could be attributed to a change in community health to care for the ill. The borderline significant association between bilateral tibial lesions and bowing may confirm this as a possibility.

As stated previously, periosteal lesions scored in the present study were generally very mild at Indian Knoll. This supports Cassidy's (1972) scoring of lesions that were possibly inflammation by Cassidy. The initial scoring system implemented for scoring PL did not adequately describe the type and severity of lesions encountered by the author, and so a scoring system was devised to account for the mildness of lesions observed. Resistance to infectious agents at Indian Knoll has been proposed (Kelley 1980:192) due to the chronic and lasting presence of infection in the sample population. Striae extending along the medial aspect of the anterior tibiae were extremely common at Indian Knoll, though not all cases presented with raised bone (i.e. new bone formation). It was very obvious upon completion of data collection for this analysis that a more aggressive infectious agent was present at Indian Knoll, and caused several causes of severe inflammatory lesions, just as Snow (1948) noted.

The prevalence of lesions recorded in the present analysis was higher than in previous studies where specific infection was noted. It is possible that the present study overestimated the prevalence of lesions by including all evidence of new bone formation, which could have included cases of trauma. Even if we total the specific and non-specific percentages of infection scored by Kelley (1980), the number of individuals affected by lesions is still lower than in the present study. However, the rates in the present study are in line with those of localized infection rates noted by Cassidy (1972:86), numbers which did not include disseminated reactions likely caused by treponemal disease. With localized infection rates topping 50 percent in middle-aged males (Cassidy 1972:86), and as low as 19 percent in young adult males, the rates observed in the present study are similar. One difference between the present study and that of Cassidy (1972) is that this analysis did not separately assess cases of more severe, diffuse inflammations indicative of treponemal disease, though they were few with regard to the entirety of the sample population analyzed.

It is also possible that some of the lesions observed in the present analysis were of a traumatic nature, thus overestimating the association between periodontal disease and periosteal lesions. However, given that both conditions were positively associated with age, it is possible that the association detected in the present study is genuine.

*Implications and Limitations of Research*

The present study sought to contribute to the discussion of systemic inflammation by identifying the associations between osteological markers of inflammation. Whereas several researchers have analyzed the prevalence of osteological lesions at Indian Knoll, the present study took this data a step further to detect associations between conditions. Several associations between lesions were detected, which may be indicative of a hyper-inflammatory state in some individuals at Indian Knoll.

While similar associations between lesions were detected in another study of archaeological remains (DeWitte and Bekvalac 2011), it must be stated that the prevalence of lesions between these populations varied. When analyzing archaeological remains, researchers are limited by the lack of definitive facts. For example, age and sex estimations are just that: estimations. Errors in estimation may lead to a distortion of the effects of age and sex on the prevalence of lesions (see Milner *et al.* 2000:475-479). Thus, the prevalence of lesions described between age categories and between the sexes may not be an accurate representation of what genuinely occurred at Indian Knoll. Additionally, the stratified random sample may have highlighted differences between age and sex since it is not a true reflection of the Indian Knoll demographic.

Additionally, caution must be exercised when comparing the associations between conditions in different populations. We must consider the two major steps in the evolution of human kind that are frequently cited in demographic studies: the advent of agriculture, and development of complex societies (Milner *et al.* 2000:471-472). Neatly categorizing archaeological populations as preceding or postdating a major event has the potential to overly generalize these prehistoric populations, and fail to discern nuances. It is for this reason that studies of a similar nature to the present one can benefit the field of anthropology: by analyzing the associations between lesions at different points in time, we may gain a better understanding of human immune responses over time, and how certain circumstances may have influenced the proliferation and severity of lesions. Additionally, analyzing populations across space, and with different social and ecological contexts, has the potential to help explain heterogeneous phenotypes when exploring systemic inflammation in skeletal remains.

# CONCLUSIONS

Perhaps the most anticipated result of this study was that of the analysis between periodontal disease and periosteal lesions, age and periosteal lesions, and age and periodontal disease. The results of the HLLA did not indicate a three-way association, and backwards elimination indicated associations between PD and age, and between PD and PL. Additionally, the chi-square analysis indicated a borderline significant association between PL and PD, though the chi-square tests between age and PL, and age and PD were positively associated with age. It is possible that a hyper-inflammatory phenotype was present in some individuals at Indian Knoll, and that both periodontal disease and periosteal lesions are good indicators to detect it. However, is also possible that an age effect is present in periosteal lesions and periodontal disease. Additionally, the scoring methods employed in the present study may have led to an overestimation of periodontal disease, and hence the association between PL and PD may be an overestimate.

As expected, abscessing was positively associated with periodontal disease. Additionally, the results of the chi-square test between periosteal lesions and bowing of the tibiae did not conclusively indicate an infectious etiology of either lesion. It is possible that tibial bowing at Indian Knoll is not due to one cause in all individuals. Perhaps cases of treponemal disease with mild bowing, in addition to cases of mild bowing from nutritional stress, are both present at Indian Knoll.

As hypothesized, childhood nutritional stress in the form of enamel hypoplasia did not significantly associate with any other lesions that could have an infectious etiology. Interesting associations were detected in individuals with exostoses, however: individuals with exostoses were more likely to exhibit porotic hyperostosis and alveolar resorption. This result could be indicative of an association between nutritional stress and inflammation. Rather, if we consider that porotic hyperostosis at Indian Knoll may have resulted from an infectious condition, the association takes on a new meaning. Additionally, the associations detected between sex and porotic hyperostosis, and sex and cribra obrbitalia, may be age related effects.

Continuing efforts to understand systemic inflammation, and how osteological markers can be used to detect it, will surely benefit the fields of anthropology and bioarchaeology. Though the present study did not attempt it, a comparative analysis between populations that share a similar time and space in history, as well as those occupying different points in time, may be able to produce a more comprehensive understanding of bone lesions and their potential role as indicators of systemic inflammation. By implementing a similar research design in other populations where Cassidy (1972) and Kelley (1980) have demonstrated a higher prevalence of some lesions than at Indian Knoll, it may be possible to see a trend in these lesions through time, and discover nuances between populations. To construct an understanding of lesion associations across time and space would take a great deal of time, necessitating a standardized methodology, but the results could give us a greater understanding of how these associations change through time and space, and how they are altered by socio-ecological factors.

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